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ECONOMIC IMPACT OF TECHNOLOGY ADOPTION IN RICE FARMING: THE CASE OF "ONE MUST DO, FIVE REDUCTIONS" IN VIETNAM

Nguyen Tien Da[1, 3, *], Vu Thi My Hue[2, 3], Hio-Jung Shin[3]

ABSTRACT

Technology adoption helps farmers gain more benefits in comparison to conventional practices. "One Must Do, Five Reductions" (1M5R) has been introduced in the Vietnamese Mekong Delta Region for nearly a decade, integrated into agricultural extension programs, and recent concerns have arisen, including farmers' low benefits and socio-economic barriers to adoption. Using survey data from 455 smallholder rice farmers, including both adopters and non-adopters, in Hau Giang province in the Winter-Spring crop 2020-2021 and Summer-Autumn crop 2021, the study confirmed the economic advantage of the adoption of 1M5R through the lower costs of production inputs and higher revenues. 1M5R adoption helped the farmers reduce 31.1-39.2% seed quantity, 10.6-16.4% nitrogen, 9.8-20.9% phosphate, 16.4-23.1% potassium in purities, and 1.67-1.84 times of pesticide spraying and significantly contributed to successfully applying AWD on rice farms, leading to an increasing net benefit of 3-5 million VND per ha. The benefit-cost ratios were estimated to increase by 35.4% and 41.4% in the Winter-Spring and Summer-Autumn rice crops, respectively. The findings of the study suggest 1M5R is a good practice in rice farming for the agricultural sector to disseminate across the Mekong Delta Region as well as to test this model in different agroecological regions through the national agricultural extension programs.

Keywords: *1M5R, economic impact, Mekong Delta Region, One Must Do Five Reductions, technology adoption.*

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1. INTRODUCTION

Rice is a staple grain that feeds the most population of the world. Rice production worldwide has reached 787.3 million tons and a harvested area of 165.3 million ha, of which the Asian region is the largest rice producer, accounting for 89.9% of the total quantity and 86.6% of the harvested area [1]. In Vietnam, the Mekong River Delta Region (MRD) accounts for 53.9% of the total harvested area, 55.5% of the total production [2], and contributes about 90% of rice export [3]. Vietnam has made great efforts in farm system reforms, hybrid rice adoption, and irrigation investment [4], and recently supported a transformation to sustainable rice production known as the "One Must Do, Five Reductions" program in the eight MRD provinces with the involvement of 104,448 smallholder rice farmers and the adopted rice area of 113,870 ha [5].

"One Must Do, Five Reductions", hereafter 1M5R, helps the MRD to improve the sustainability of rice production by reducing major inputs such as chemical fertilizers and pesticides and then reducing negative impacts on the environment [6]. From an economic perspective, 1M5R helps smallholder farmers increase their profits in comparison to conventional rice farming [5]-[7]. The increase in rice-farming profitability relies on increased rice yield through the intensive use of production inputs such as seeds, fertilizers, pesticides, and labor. However, overuse of production inputs, at some point, has been reduced profits and caused serious consequences for health, the environment, and sustainable agriculture. Some challenges in reducing chemical fertilizers, irrigated water, and seed as well as low income in rice farming reported recently in some MRD provinces, alert the maintenance of 1M5R in the region [8], [9]. Therefore, the objective

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of this study is to revisit the economic impact of 1M5R with the hypothesis that 1M5R adoption helps farmers gain more benefits compared to conventional rice farming. The relevant environmental issues have also been discussed in the following.

2. RESEARCH METHODS

2.1. 1M5R definition

In 2012, the Department of Crop Production, the Ministry of Agriculture and Rural Development of Vietnam (MARD) recognized 1M5R as an advanced technical package in rice farming by Decision No. 532-QD-TT-CLT dated on November 7, 2012. Historically, 1M5R has been developed through longitudinal technical support from the International Rice Research Institute (IRRI) and a national expert panel based at the Plant Protection Department (PPD) of An Giang province through some technology transfer projects [10]. 1M5R in nature requires simultaneous activities in rice farming. Specifically, "One Must Do" requests the usage of seeds certified by the authorized organizations meanwhile requests reduction in seed rate, use of fertilizers and pesticides, irriation cost, and post-harvest losses, farmland management following the Alternate Wetting and Drying technique (AWD) as well as using combine harvesters. In our study, a farmer is defined as an adopter if he or she meets all criteria of 1M5R below. If at least one criterion unqualifies, the farmer is defined as a non-adopter [11]. The criteria for the 1M5R assessment are presented in table 1.

Table 1. Description of 1M5R criteria

Criteria	Non-adoption	Adoption
Seed standard	Uncertified	Certified
Seed rate (kg ha^{-1})	>120	80 -120
Nitrogen (kg ha^{-1})		
Winter - Spring crop	> 100	≤ 100
Summer - Autumn crop	> 80	≤ 80
Pesticides (times per crop)	>5	≤5
AWD application	No	Yes
Combine harvester application	No	Yes

2.2. Data collection and processing

A total of 455 rice farmers, including farmers who adopted 1M5R and farmers who did not adopt 1M5R, participated in the survey. The selected areas represent the different soil conditions, a critical regulation in Decision No. 532-QD-TT-CLT [10], in Hau Giang province (Table 2). The study used a structured questionnaire asking about the household farmers' characteristics and their rice farming practices in the Winter-Spring rice crop in 2020-2021 (W-S) and the Summer-Autumn rice crop in 2021 (S-A). The missing data was fulfilled by the responsible interviewees, who are the district and communal agricultural extension staff belonging to the Department of Agriculture and Rural Development (DARD) of Hau Giang province and had previously been trained for data collection.

The surveyed information was transformed into an Excel sheet, and some calculation steps were conducted to convert the input information; for example, compound fertilizers and single-element fertilizers need to be transformed into pure nitrogen, phosphate, and potassium. Next, the data processing, including calculation and testing, was performed by Stata software, Version 17.

Table 2. The surveyed area for 1M5R data collection

District	Commune	Number of households	Soil condition
Vi Thuy	Vi Thang	93	Alluvial
Vi Thanh	Vi Tan	116	Acid
Long My	Xa Phien	103	Saline
	Thuan Hung	113	Saline
Long My town	Long Tri	30	Saline
Total		455	

2.3. Economic analysis

The economic variables include the costs of the production inputs, which are seeds, fertilizers, pesticides, pumping, labor, and harvesting; and the other costs, which include public irrigation fees and property depreciation; and the outcomes, which include rice revenue and other revenue. Net benefit (π) is calculated as total revenue (TR) minus total costs (TC). The benefit - cost ratio (BCR) is the ratio between the net benefit (π) and the total costs (TC). Equations (1) and (2) illustrate their calculations, respectively.

$$\pi = TR - TC \qquad (1)$$

$$BCR = \frac{\pi}{TC} \qquad (2)$$

All the currency indicators are calculated in thousand Vietnamese Dong. For testing, the two-sample t-test was used to determine whether the means of two groups are equal or not.

3. RESULTS AND DISCUSSION

3.1. Rice production in Hau Giang province

Hau Giang province is located at the center of the MRD and administratively formed of 1 city, 2 towns, and 5 districts. The natural land covers 162.2 thousand ha, of which agricultural land is approximately 134 thousand ha and ranked ninth amongst MRD provinces [12]. The rice farming system covers 2 rice crops, some areas produce 3 rice crops or rice-fish rotation. In recent years, the total annual rice area of Hau Giang has fluctuated around 190,000 ha, with the average production and productivity at 1,250,000 tons and 6.4 tons ha-1, respectively (Figure 1) [2].

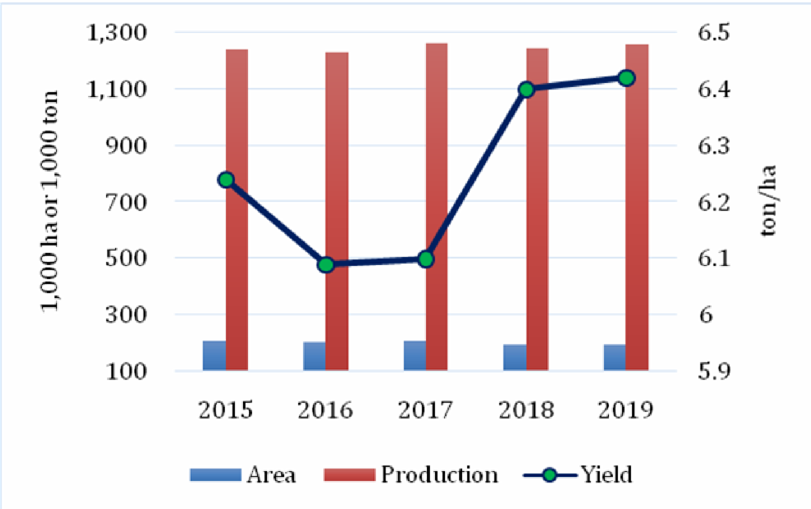


Figure 1. Rice production of Hau Giang province from 2015 - 2019

3.2. Characteristics of the participants

In our study, 68-69% of the farmers adopted 1M5R, a far more impressive proportion than a prior estimate of 40% in the other MRD province [13]. Almost the adopters (97-99%) participated in agriculture cooperatives, while only 34-38% of non-adopters were the cooperative members. Besides, 71-73% of the adopters were male farmers because men often manage agriculture farms as well as the targets of extension programs [14], [15]. For the non-adopter group, only 49-53% were male farmers. The average age of the adopters was 50-year-old, significantly 3-year-old younger than the non-adopters. This result is consistent with many other findings that young farmers are more likely to adopt new technologies [16]–[18]. For education attainment, the education level of the two farmer groups did not significantly differ in our study. The adopters and non-adopters attended on average 6.55-6.72 and 6.03-6.38 years, respectively, in the 12-year schooling system in Vietnam. Education attainment is a controversial factor in technology adoption literature. Well-educated farmers tend to actively adopt agricultural technologies [19], [20] but in some cases, the education level of farmers does not determine their adoption behavior [21], [22]. Most of the adopters (93%) and non-adopters (88 - 89%) were Kinh, the majority ethnic group in Vietnam, and the rest were Khmer. There was no significant difference in the proportion of Kinh group between the adopters and non-adopters in S-A although it was significantly different in W-S and Kinh is reported a higher probability of technology adoption [23].

Table 3. Characteristics of the participants

Variables	W-S			S-A		
	Adopters n = 312	Non-adopters n = 143	Mean difference	Adopters n = 309	Non-adopters n = 146	Mean difference
1M5R adoption	0.69	0.31	-	0.68	0.32	-
Cooperative member	0.99 (0.11)	0.34 (0.48)	0.65***	0.97 (0.16)	0.38 (0.49)	0.59***
Gender	0.73 (0.44)	0.49 (0.50)	0.24***	0.71 (0.45)	0.53 (0.50)	0.18***
Age	49.62 (12.22)	52.79 (11.13)	-3.17***	49.68 (12.27)	52.61 (11.09)	-2.93***
Education	6.55 (2.79)	6.38 (2.77)	0.17 ns	6.72 (2.70)	6.03 (2.89)	0.69***
Ethnicity	0.93 (0.26)	0.88 (0.32)	0.05***	0.93 (0.26)	0.89 (0.31)	0.04 ns

*Note: Standard deviation in parenthesis; Significance level: *** = 1%, ** = 5%, * = 10%, No significance = ns; Unit of variables: 1M5R adoption: 1 = adopted, 0 = otherwise; Cooperative member: 1 = yes, 0 = otherwise; Gender: 1 = Male, 0 = Female; Age: The age of household header; Education: The education level of household header; Ethnicity: 1 = Kinh, 0 = otherwise*

3.3. Rice farming characteristics and environmental impacts

The adopters cultivated an average of 0.90-0.94 ha rice farmland, while the non-adopter farmed on an area of 0.79 - 0.85 ha. These farm sizes are impressive in comparison to half of the MRD households cultivating less than 0.5 ha [24]. No difference was reported in the rice area between the adopters and non-adopters in W-S, but the adopters cultivated on a significantly 0.15 ha higher area than the non-adopters in S-A.

As regards the main production inputs, including seeds, fertilizers, and pesticides, the non-adopters invested a more significant amount than the adopters in the two rice crops. The non-adopters used an extra 30-39 kg ha-1 seeds than the adopters, equivalent to 31.1-39.2% higher in comparison to the adopters' usage. Regarding fertilizers, the problem of excessive usage is reported to negatively impact on pests, diseases, and underground water. In our study, the non-adopters used an extra 10.61-13.08 kg ha-1 nitrogen than the adopters did. In other words, the non-adopters overused 10.6-16.4% nitrogen in W-S and S-A, respectively. Similarly, the non-adopters used an extra 5.23-11.57 kg ha-1 phosphate, equivalent to 9.8% and 20.9%, and 6.34-7.65 kg ha-1 potassium, equivalent to 16.4% and 23.1%, compared to the adopters' usage. Although the 1M5R assessment does not include phosphate and potassium thresholds, but the increase in nitrogen will lead to an increase in the usage of phosphate and potassium on rice farms. As a result, overuse of all fertilizers and more diseases occur on the rice farms. Indeed, the non-adopters sprayed pesticides during both rice crops on rice farms more than the adopters 1.67-1.84 times. This might lead to intensive labor and chemicals utilized, that we examine in the next section.

As regards the rice yield, in general, the adopters achieved 7.84 tons ha-1 in W-S and 5.65 tons ha-1 in S-A meanwhile the non-adopters achieved 7.63 tons ha-1 and 5.58 tons ha-1, respectively. The farmers in our study achieved a higher yield in comparison to the average rice productivity in MRD, which yielded only 6.83 tons ha-1 in W-S and 5.56 tons ha-1 in S-A [2]. Our study found that in W-S, the adopters yielded 0.21 tons ha-1, equivalent to 2.8%, significantly higher than the non-adopters while utilizing lower main production inputs. This result shares the common finding with some corresponding research [6], [7], [25] and affirms the overuse of production inputs in the MRD. In S-A, the non-adopters were reported to yield 0.2 tons ha-1, equivalent to 3.5%, significantly higher than the adopters. The S-A in the MRD region is well known for the influence of harsher natural conditions such as high temperature and heavy rainfall, so production inputs might play a decisive role in rice yield.

The literature often discusses the environmental advantages associated with technology adoption as a bonus of the adoption behavior rather than environmental outcomes as well as human health alone. Therefore, these issues are not given sufficient discussion in rice farming. In MRD, the farmers often use knapsack sprayers to spray pesticides in the absence of appropriate protection equipment such as masks, gloves, and protective clothing [26]. When 1M5R is adopted, the reduction in pesticides directly affects, in a positive manner, to the health of farmers when spraying these toxic chemicals on rice fields. The reduction in fertilizers such as nitrogen (10.61-13.08 kg ha-1), phosphate (5.23-11.57 kg ha-1), and potassium (6.34-7.65 kg ha-1) creates a large spillover effect when 1M5R has been disseminated across the MRD provinces, promising a reduction in water underground pollution at the regional level. In Vietnam, the agricultural sector emitted 104.5 Mt CO_2eq, of which rice production accounted for 41.9 Mt CO_2eq, equivalent to 40% [27], and 1M5R adoption helped reduce GHG emissions by 26.6% and 29.9% in W-S and S-A, respectively [28].

Table 4. Characteristics of rice farming

Variables	W-S			S-A		
	Adopters n = 312	Non-adopters n = 143	Mean difference	Adopters n = 309	Non-adopters n = 146	Mean difference
Area	0.90 (0.72)	0.85 (0.60)	0.05 ^{ns}	0.94 (0.73)	0.79 (0.56)	0.15***
Seed rate	96.57 (7.10)	126.61 (27.97)	-30.04***	98.76 (3.29)	137.45 (32.59)	-38.69***
Nitrogen rate	79.33 (10.93)	89.94 (21.01)	-10.61***	80.74 (11.41)	93.82 (27.39)	-13.08***
Phosphate rate	55.37 (13.26)	66.94 (17.77)	-11.57***	53.17 (10.39)	58.40 (22.37)	-5.23***
Potassium rate	33.11 (11.01)	45.77 (29.16)	-7.65***	38.69 (9.52)	45.03 (32.63)	-6.34***
Spraying	5.90 (1.31)	7.75 (2.58)	-1.84***	6.05 (1.25)	7.72 (2.04)	-1.67***
Yield	7.84 (0.44)	7.63 (0.40)	0.21***	5.65 (0.49)	5.85 (0.42)	-0.20***

*Note: Standard deviation in parenthesis; Significance level: *** = 1%, ** = 5%, * = 10%, No significance = ns; Unit of variables: Area: ha; Seed rate: kg ha-1; Nitrogen rate, Phosphate rate, and Potassium rate: pure kg ha-1; Spraying: times crop-1; Yield: t ha-1*

3.4. Economic analysis of rice farming

Table 5 indicates the cost-benefit analysis and BCR indicator between the two farmer groups. The total cost covers all the component costs incurred during rice farming, including production costs and other costs. In our study, family labor cost was calculated according to the average hired labor in the local area. The other cost might not cover all its components because, in our study, other cost was calculated based on only the cost of depreciation of irrigation systems and properties. However, we assume the remaining other costs only account for a small proportion. Total revenue equals the revenue from selling rice plus other revenue, which comes from selling by-products such as straw and husk.

As regards the costs, our study shows that the non-adopters paid significantly more than the adopters for all the input costs, except for land preparation in W-S, and for pumping and hired labor in S-A. Overall, the adopters had to pay approximately 13 million VND in W-S and 13.3 million VND in S-A meanwhile the non-adopters had to pay approximately 15 million VND and 16 million VND in the corresponding rice crops. The total costs the non-adopters paid more than the adopters by approximately 2.0 million VND in W-S and 2.8 million VND in S-A, equivalent to 15.2% and 20.9% of the total production costs, respectively. Figure 3 shows the illustration for the input costs, highlighting the difference in the costs of seeds, fertilizers, and pesticides between the two farmer groups. There is no doubt that the non-adopters used more major inputs than the adopters, and as a result, more cost they had to pay. An interesting point in the input costs is that the adopters paid significantly higher costs for family labor in W-S, for land preparation and family labor in S-A than the non-adopters. It can be explained that the adopters paid more for land preparation because they might flatten more their rice fields in order to easily apply AWD, a mandatory criterion in 1M5R practice. The adopters paid more for family labor partly because they had to spend more working days visiting the rice farms. More farm visits are popular in other sustainable rice farming practices, for example, Integrated Pest Management (IPM) where the farmers regularly observe the ecosystem during the crop [29]. For other costs, the non-adopters paid 133.40 - 195.79 thousand VND more than the adopters did. In this case, the difference in other costs, on the one hand, was explained that the non-adopters had to pay more on property depreciation because the MRD farmers often calculate this cost based on the amount of seed sown. On the other hand, the cost of irrigation is noted to be equal for each hectare in the same area. These contribute to the difference of other cost of the two farmer groups.

Table 5. Economic indicators of rice farming (1,000 VND)

Variables	W-S			S-A		
	Adopters n = 312	Non-adopters n = 143	Mean difference	Adopters n = 309	Non-adopters n = 146	Mean difference
Costs						
Seed	1,512.50	1,819.08	-306.58***	1,412.91	1,802.87	-389.96***
Land preparation	865.32	837.42	27.90 ns	1,058.53	1,002.87	55.66***
Fertilizers	3,331.28	3,816.01	-484.73***	3,122.14	3,667.29	-545.15***
Pesticides	2,531.03	3,238.36	-707.33***	2,780.92	4,407.98	-1,627.06***
Pumping	655.88	731.43	-75.55***	638.58	631.91	6.67 ns
Harvesting	2,052.00	2,190.52	-138.52***	2,046.94	2,171.47	-124.53***
Hired labor	1,028.36	1,213.14	-184.78***	1,208.79	1,281.85	-73.06 ns
Family labor	146.88	64.34	82.55***	134.63	79.45	55.18***
Other cost	865.97	1,061.76	-195.79***	867.62	1,001.02	-133.40***
Total cost	12,989.22	14,972.06	-1,982.84***	13,271.06	16,046.72	-2,775.67***
Revenues						
Rice revenue	43,574.31	40,464.27	3,110.03***	29,782.56	29,778.77	3.79 ns
Other revenue	75.72	117.03	-41.31***	360.70	115.76	244.94***
Total revenue	43,650.03	40,581.30	3,068.73***	30,143.26	29,894.53	248.73 ns
Benefit						
Net benefit	30,660.81	25,609.24	5,051.56***	16,872.21	13,847.81	3,024.40***
Ratio						
BCR	2.45	1.81	0.64***	1.33	0.94	0.39***

*Note: Significance level: *** = 1%, ** = 5%, * = 10%, No significance = ns.*

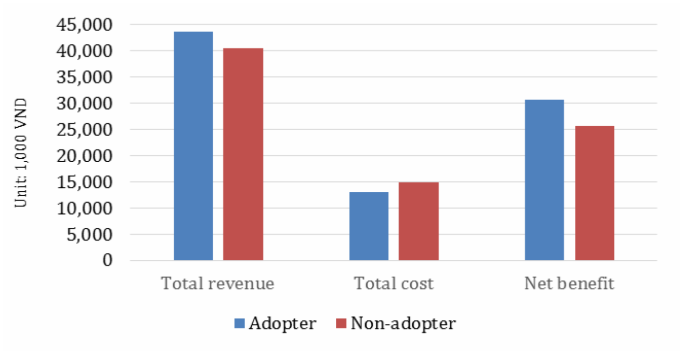
As regards the revenues, in general, the adopters achieved approximately 43.7 million VND in W-S and 30.1 million VND in S-A meanwhile the non-adopters achieved 40.6 million VND and 29.9 million VND, respectively. Our study found that in W-S, a significant difference of approximately 3.1 million VND in total revenues between the adopters and the non-adopters was recorded. The reason behind this is the majority contribution of significant difference in rice revenue. However, the total revenue in S-A was estimated to be equivalent between the two farmer groups. Compared to the non-adopters, the adopters obtained the same rice revenue and approximately 0.24 million VND higher in other revenue. Nevertheless, the higher difference in other revenue was not enough to make the total revenue different. It is worth noting that, on average, the total revenue in W-S varied from 40.6-43.7 million VND ha-1, approximately 1.4-1.5 times higher than the total revenue in S-A, which varied from 29.9-30.1 million VND ha-1. In other words, W-S generated a higher economic benefit for MRD farmers than S-A did, thanks to its higher total revenue (Figure 2).

As regards the net benefit, the adopters achieved the net benefits of approximately 30.7 million VND ha-1 in S-W and 16.9 million VND ha-1 in S-A meanwhile the non-adopters achieved approximately 25.6 million VND ha-1 and 13.8 million VND ha-1, respectively. Our study found the adopters achieved significantly more net benefit than the non-adopters approximately 5 million VND in W-S and 3 million VND in S-A. Compared to the non-adopters, the higher net benefit of the adopters stemmed from the lower total cost and higher total revenue in W-S, and the lower total costs in S-A. This result is consistent with a study in Thailand where the farmers obtain higher profits by saving the total cost of fewer production inputs [25].

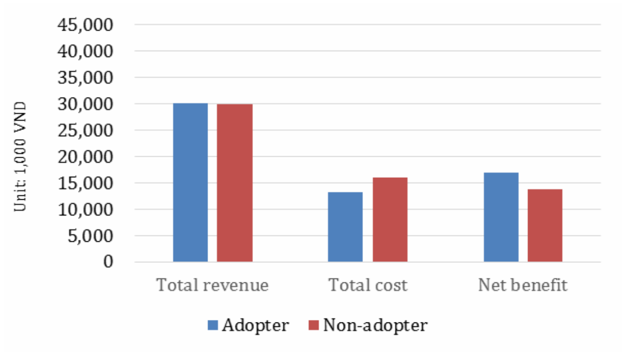
In our study, we employed a very simple form of benefit-cost ratio because the main purpose of the study is to compare the farming practices between the two groups of farmers within the same crop. Benefit-cost ratios explain economic efficiency in economic activities, and our estimated BCRs particularly reflectect the superiority of the net benefit against the total cost (see Equation 2). Our study found significant differences in BCR between adopters and non-adopters in both two crops. In W-S, the BCR of the adopters was 2.45, significantly higher than that of the non-adopters at 1.81. Similarly, in S-A, the BCR of the adopters was 1.33, significantly higher than the non-adopters' BCR of 0.94. In other words, 1M5R adoption increased BCRs by 35.4% and 41.4% in W-S and S-A, respectively and both were higher than 28%, as reported in Can Tho city in 2015 [6]. The BCRs in our study confirmed the economic advantage of 1M5R compared to conventional rice farming, and the higher differences in BCRs between two crops, W-S and S-A, indicated the importance of W-S in the MRD rice farming system.

4. CONCLUSION

Rice farming plays a crucial role in the MRD, contributing to Vietnam's food security and exports. Technology adoption in rice farming generally provides higher yields at farm scale but does not automatically generate maximum net benefits for smallholder farmers. The existing literature has identified 1M5R as a profitable model that meets the requirement to shift MRD agriculture from intensive-input production to rice economics. Recent reported challenges in reducing inputs and farming practices prevent MRD agriculture from disseminating of 1M5R across the region. In this study, we used the survey data collected in Hau Giang province to examine the economic perspective of 1M5R adoption at the smallholder farmer scale. The result of the economic analysis confirmed that 1M5R adopters achieved greater significant profitability than the non-adopters, thanks to not only lower expenses for some major production inputs, including seeds, fertilizers, and pesticides, but also higher total revenue. Our study estimated that 1M5R adoption helped the farmers reduce 31.1-39.2% seed quantity, 10.6-16.4% nitrogen, 9.8-20.9% phosphate, 16.4-23.1% potassium in purities, and 1.67-1.84 times of pesticide spraying and successfully applied AWD on rice farms, leading to a higher net benefit from 3-5 million VND ha-1 than non-adoption. The adoption therefore increased BCRs by 35.4% and 41.4% in W-S and S-A, respectively. Our findings suggest the agricultural sector should continue the dissemination of 1M5R across the MRD provinces as well as test this model in different agroecological regions through the national agricultural extension programs.

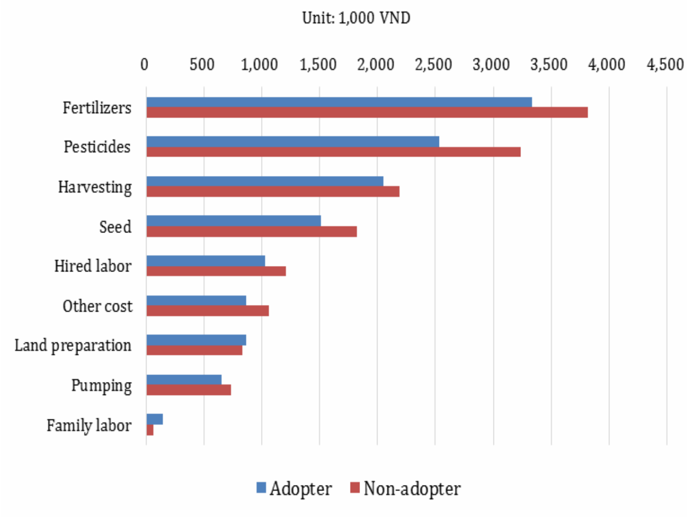


W-S

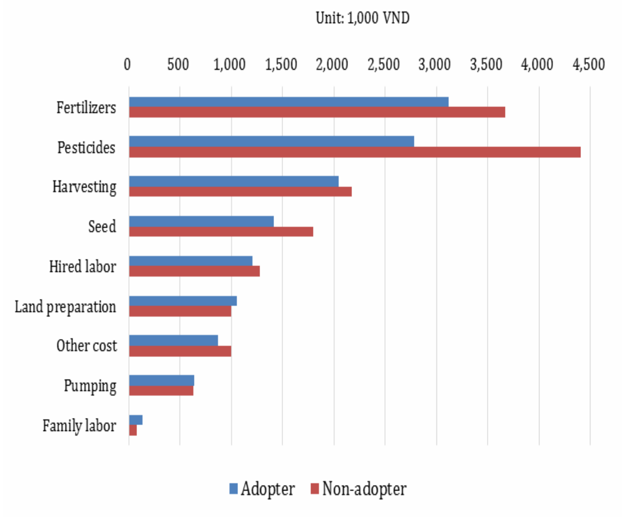


S-A

Figure 2. Cost-Benefit analysis (CBA) between the two farmer groups



W-S



S-A

Figure 3. The costs of rice farming between the two farmer groups

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AUTHORS' CONTRIBUTION

N.T.D contributed to the design, analysis, and implementation. V.T.M.H contributed to the design and analysis. H.J.S. contributed to the design. All the authors jointly participated in the manuscript writing.

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RESEARCH ON GROWTH, YIELD AND QUALITY CHARACTERISTICS OF IMPORTED ASPARAGUS VARIETIES ADAPTED TO THE RED RIVER DELTA, VIETNAM

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ABSTRACT

Asparagus (*Asparagus officinalis* L.) is known as a valuable vegetable due to its richness in anti-cancer and antioxidant compounds, vitamins, minerals, and high economic efficiency. Asparagus is being expanded in production in Vietnam. Breeding asparagus varieties has not been carried out in Vietnam, so asparagus varieties grown in Vietnam are all imported varieties. The study aimed to choose the best varieties among 12 asparagus varieties imported from the US and Netherlands for production in the Red River Delta, Vietnam. The yield of spears was assessed in the spring and autumn season 2022 using the second-year asparagus in the field of the Field Crops Research Institute, Hai Duong province, Vietnam. The results indicated that Atlas F1 was the variety with the most advantages in both seasons, with a low infection rate of stemblight, large diameter of spears, high dry weight of spears, high yield and yield component, and moderate vitamin C content. In addition, Purple passion was also a special variety with high stability due to its low infection rate, high dry weight, high vitamin C content in both seasons, high sugar accumulation rate in roots, root CHO, and total sugar content in the autumn season 2022, although the yield productivity and yield component were low. The results also showed a positive and significant correlation between asparagus yield productivity and root Brix in both seasons of 2022. The results suggest that the root Brix of asparagus could be the target trait for choosing varieties for production.

Keywords: *Asparagus officinalis, asparagus varieties, root Brix, root CHO, quality, yield.*

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1. INTRODUCTION

Asparagus (*Asparagus officinalis* L.) belongs to the family *Asparagaceae* and is a dioecious perennial crop. Asparagus is a premium vegetable with edible shoots called spears [1]. Asparagus has been grown in 45 countries around the world, on five continents: Europe, America, Asia, Africa, and Australia. The average worldwide yield in 2021 was 5.33 tons/ha. The total area for harvest in 2021 worldwide is 1.60 million hectares, with China taking the leading position at 1.44 million hectares [2].

Asparagus products are rich in anticancer and antioxidant compounds such as rutin, saponins, polyphenols, polysaccharides, and polyamines, which are considered high-value products [3]. Asparagus is rich in vitamins, minerals, and folate salts: potassium (202 g/100 g fresh), folate (52 mg/100 g fresh), vitamin A (38 mg/100 g fresh), C 95.6 mg/100 g fresh), K (41.6 mg/100 g fresh), phosphorus (52 mg/100 g fresh), calcium (24 mg/100 g fresh), magnesium (14 mg/100 g fresh), and very little sodium (2 mg/100 g fresh) [4].

In Vietnam, the first asparagus was planted in 1960. Until 2005, asparagus was widely grown in Ninh Thuan, Binh Thuan, Ba Ria - Vung Tau, Dong Nai, Cu Chi, Binh Phuoc, Vinh Long, and An Giang [5]. Recently, asparagus has been grown in the North: Hai Duong, Ha Noi, Hai Phong, Bac Ninh, and Phu Tho. Asparagus can bring a high income to

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growers. In Ninh Thuan province, farmers earn 150-300 million VND/ha/year from asparagus. Prices range from 30,000 VND to 90,000 VND per kg depending on the season. This crop has been considered a high-value crop [5].

In the production of asparagus, the first strategy is to choose a source of asparagus varieties adapted to the growing regions. Additionally, the cultivation must remain productive for a few years to recover the initial investment and make a profit [6]. However, the morphological characteristics and yield of asparagus depend on the characteristics of different varieties, techniques, and growing conditions [7]. Asparagus breeding has not been done in Vietnam, so seeds are imported mainly from the US and the Netherlands [8]. In recent years, some heat-resistant asparagus varieties imported from the Netherlands and the US have been tested in some regions [8], [9]. However, choosing a suitable variety of asparagus for the Red River Delta-the center of Vietnam's socio-economic development, is essential. Therefore, this study aims to select the promising varieties imported from the US and Netherlands for asparagus production in the Red River Delta in Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

Twelve varieties of F1 asparagus were imported from the US and Netherlands, which are listed in table 1.

Table 1. List of asparagus varieties participating in the experiment

No	Varieties	Origin	No	Varieties	Origin
1	K767	Netherlands	7	UC157	USA
2	Starlim	Netherlands	8	Grande	USA
3	Lunalim	Netherlands	9	WB212	USA
4	Apollo	USA	10	WB230	USA
5	Atlas	USA	11	WB231	USA
6	Delux	USA	12	Purple passion	USA

2.2. Methods

2.2.1. Experiment design

The experiment was arranged in a completely randomized block design (RCBD), with 12 varieties and 3 replicates in the open field at Field Crops Research Institute, Hai Duong province, Vietnam, with the attitudes of 20°53' N and 106°17' E. The experimental plot size is 25 $m^2 plot^{-1}$, planting 2 $rows^{-1}$, planting distance 0.75 m x 0.4 m. The experiment was planted on March 27, 2020. Seedlings were planted when 3 stems per plant were 3 months old. The second-year asparagus was evaluated in this study. The experiment was evaluated in two seasons (spring season 2022 and autumn season 2022) of the second asparagus year. Soil was sampled in the second year of asparagus to analyze its chemical properties (Table 2).

Table 2. Soil chemical properties - sampling at the second-year asparagus

pH	OC	N	P_2O_5	K_2O	N	P_2O_5	K_2O	Ca	Mg	Fe
	%				mg/100 g			mg/100 g		
7.69	1.54	0.12	0.32	2.07	5.2	33.1	21.4	274.7	22.2	18.5
Rating hierarchy/Threshold										
High	>2,0	>0,2	>0,1	>2,0	>6	>10	>20	>160	>36	-
Low	<0.9	<0,1	<0,06	<1,0	4-6	<5	<10	<40	<12	-
Moderate	1,0-1,9	0,1-0,2	0,06-0,10	1,0-2,0	<4	5.0-10.0	10-20	80-160	12-36	-

Note: Fertilizer for the second-year asparagus: Organic fertilizer: 40 tons/ha; NPK: 150 kg N: 150 kg P_2O_5: 150 kg K_2O ha; MgO: 30 kg/ha.

2.2.2. Asparagus growth, yield measurements

Growth characteristics: Diameter dynamics of harvested spears were measured from emergence to harvest height (spear length greater than 27 cm). The size of the sample was 10 plants per replicate per variety. The chlorophyll content of a fully expanded leaf was measured by the method of Wintermans and De Mots A (1965) [10]. Carbohydrate content (CHO) in the root was measured using the Wilson *et al.* (2008) [11] method via root Brix: Dug 50 cm depth, took 3 roots/cluster, then cut 15 cm roots in the middle. Root extract was measured Brix with a Brix meter.

Water loss was determined on a fresh weight basis. For each treatment, ten spears per replication were harvested separately, and the fresh weights were recorded immediately. The spears were kept in normal room conditions (26°C ± 1°C temperature, 75% ± 3% relative humidity) for 6 hours. After 6 hours, the spears were weighed for calculate spear moisture loss. Then, these spears were dried in the oven at 80°C for many hours until a constant weight was reached and the dry weights were recorded.

The spear moisture content (SMC) and spear moisture loss of the treatments were calculated separately using the following formulas and expressed as percentages (%):

$$\text{Spear moisture content (\%) (SMC)} = \left[\frac{\text{Fresh weight of spear} - \text{Dry weight of spear}}{\text{Fresh weight of spear}} x\ 100\right]$$

Water loss = 100% - SMC

Stem blight *Phomopsis asparagi* infection was surveyed in May and autumn season 2022, from the end of the rainy season: *Phomosisasparagi* infection rate was measured based on the number of infected plants in a total of 20 plants of each replication per variety with the following formula:

Disease rate = number of infected stems/total number of stems in the cluster

Asparagus yield of the second asparagus year in the spring and autumn seasons, 2022: each season harvested for 30 days. The length of the harvested spear was 27-30 cm.

Quality traits of spears: Brix of spears was measured using the Brix meter (Milwaukee MA882); Nitrate content in spears (NO_3^- content) was measured using the SoeksNuc 091-01 machine; Vitamin C content (mg $100g^{-1}$), Fiber content (%) [12] and Total sugar content (%) in spear were measured as per protocols described previously [12-14].

Data analysis

Analysis of variance (ANOVA) was performed using Minitab 16 software, and the significance of mean values was analyzed by Tukey's test.

3. RESULTS

3.1. Climate condition of the experimental site

The climate in Hai Duong province has been considered representative of the climate of the Red River Delta, Vietnam, and subtropical regions (Figure 1). The spring season started from January to March with low temperatures and rainfall. Next was summer (April to July) and autumn season (August to September), in which August was the heaviest rain and a favorable condition for stem blight disease to thrive. December-winter months dropped sharply in temperature and rainfall in a year.

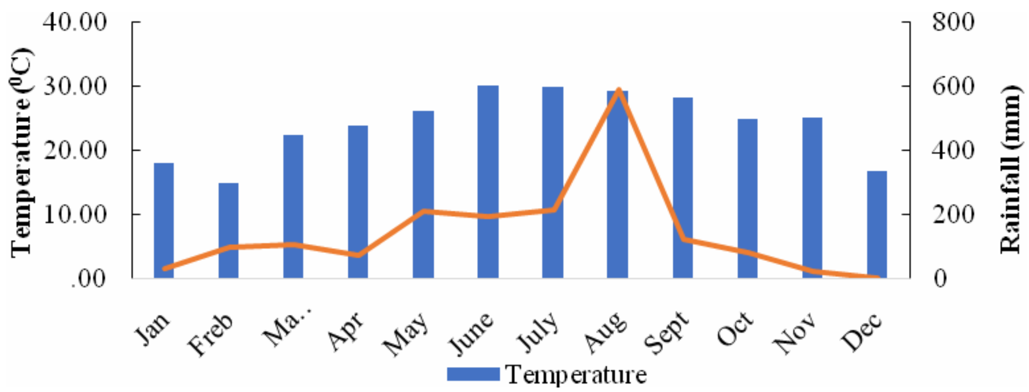

Figure 1. Mean monthly climate data of Hai Duong province in 2022

Source: Hai Duong hydrometeorological station 2022.

3.2. Growth characteristics of spears of imported asparagus varieties

The diameters of spear bases measured at peak harvest reflect the spear thickness of each variety. The diameter of spears increased gradually from day 1 to day 3, and 4, some varieties reached the harvested diameter on day 4. The diameter of spears in the spring season of 2022 tended to be higher than in the autumn crop season of 2022 (Figure 2).

In the spring season of 2022, on day 1 and day 2, the difference in diameter of spears was not statistically significant, but from day 3 to day 4, different varieties gave significantly different diameters of spears. The highest and most significant difference in the diameter of spears was Atlas and K767 varieties. Varieties with spear diameters ranged from 0.55 to 0.94 cm (Figure 2).

In the autumn season of 2022, the varieties with a high spear diameter and significantly different from the remaining varieties on day 3 were Grande, Atlas, Purple passion, UC157, and K767. The spear diameter of asparagus varieties ranged from 0.66 - 0.85 cm (Figure 2).

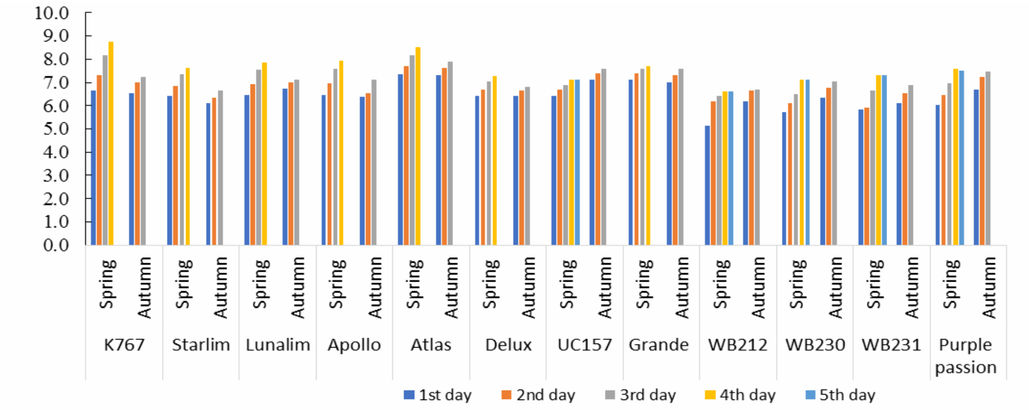

Figure 2. Diameter dynamics of harvested spears of imported asparagus varieties in the spring and autumn seasons of 2022

3.3. Physiological characteristics of spears of imported asparagus varieties

The root Brix was measured before harvest. The root Brix of varieties in the spring season of 2022 was higher than that in autumn 2022; in both seasons, the Atlas variety still achieved a high root Brix and was significantly different from the other varieties. In the spring season, root Brix was highest in the Atlas variety and was not significantly different from UC157, Grande, K767, Delux, and lowest in the WB212, WB 230, and Apollo varieties. In the autumn season of 2022, root Brix was highest in Purple passion and was not significantly different from Atlas, Grande, and lowest in Lunalim (Table 3).

Table 3. Root Brix, root carbohydrate, and chlorophyll content of imported asparagus varieties

Variety	Root Brix (%)		CHO (mgg^{-1})		Chlorophyll in Autumn 2022		
	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022	Chl. a	Chl. b	Chl. a+b
K767	18.88abc	15.0bc	441.3abc	377.4ab	28.9ab	63.7ab	92.3ab
Starlim	16,33def	14.5bc	387.5def	349.6bc	28.9a	63.0ab	91.5ab
Lunalim	17.75bcde	11.1d	419.6bcde	298.2d	29.8a	59.7ab	89.2ab
Apollo	14.77f	15.2b	354.5f	364.3abc	28.5ab	63.9ab	92.1ab
Atlas	20.77a	15.6ab	481.1a	385.4a	28.8ab	63.0ab	91.5ab
Delux	18.33abcd	15.0bc	440.3abcd	360.1abc	29.2ab	62.6ab	91.5ab
UC157	19.23ab	15.0bc	448.7ab	360.1abc	29.3ab	62.5ab	91.5ab
Grande	18.92ab	15.6ab	469.4ab	372.8ab	28.8ab	63.4ab	91.91ab
WB212	14.83f	14.0c	355.9f	339.0c	29.0ab	62.9ab	91.6ab
WB230	14.83f	14.5bc	355.9f	348.1bc	29.5ab	59.7ab	88.9b
WB231	15.07ef	14.5bc	360.8ef	3448.1bc	29.4ab	59.6b	88.7b
Purple Passion	16.37cdef	16.4a	388.2cdef	384.0a	28.5b	65.3a	93.4a

Note: Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

CHO content in the root was also higher in the spring season than in the autumn season of 2022 because the root Brix in the spring season was higher than in the autumn season. In both seasons, the Atlas variety had the highest CHO content. In the spring season of 2022, the Atlas variety had the highest CHO, followed by UC157, Grande, and K767. In the autumn season of 2022, the Atlas variety also had the highest CHO, followed by Purple assion and Grande.

Chlorophyll a + b in the autumn season of 2022 was highest in the Purple passion variety and lowest in the WB231 variety. However, all varieties had no significant difference in the amount of chlorophyll a + b in leaves except for two varieties WB230, which was significantly lower than the remaining varieties (Table 3).

The Spear moisture content of spears did not differ between varieties in both seasons (spring and autumn), ranging from 92.23-95.35% in spring 2022 and 90.3-92.1% in autumn season 2022. Spear moisture content in the spring season of 2022 tended to be higher than in the autumn season. However, the spears moisture loss after 6 hours of the autumn season 2022 tended to be higher than that of the spring season (Table 4).

Table 4. Spear moisture content, spear moisture loss after 6 hours, and dry weight of imported asparagus varieties

Variety	Spear moisture content (%)		Spears moisture loss after 6 hours levels (%)		Dry weight (g/100 g fresh weight)	
	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022
K767	93.56a	90.8a	7.36b	7.9a	6.51cdef	9.2ab
Starlim	94.46a	91.7a	9.7a	7.2a	6.02f	8.0bc
Lunalim	93.31a	91.4a	5.0efg	8.2a	6.40cdef	8.2bc
Apollo	93.77a	92.3a	5.5de	7.7a	6.11ef	7.9bc
Atlas	92.69a	90.3a	5.1efg	8.4a	7.41a	9.8a
Delux	92.87a	90.7a	5.9cd	7.6a	6.90abc	8.1bc
UC157	93.42a	92.1a	6.2c	7.5a	6.78bcd	8.9abc
Grande	94.24a	90.9a	5.0efg	8.2a	6.39cdef	9.1abc
WB212	92.23a	91.6a	5.0fg	6.3a	6.58bcde	8.4abc
WB230	95.35a	90.4a	6.1c	6.8a	6.53bcdef	8.7abc
WB231	93.66a	91.2a	5.3ef	7.5a	6.35dè	7.6c
Purple passion	93.3a	90.7a	4.7g	6.7a	7.1ab	9.0abc

Note: Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

The dry weight of the autumn season was higher than that in the spring season, and the Atlas variety had the highest dry weight in both seasons. The varieties with high dry weight in both crops are Purple passion, Grande, and K767 (Table 4).

3.4. Phormosis asparagi infection rate of some asparagus varieties in the autumn season 2022

Table 5. The situation of Phomopsis disease of asparagus varieties in the autumn season of 2022

Varieties	Infection rate (%)			
	May	August	September	October
K767	26.9	56.9	63.2	64.65
Starlim	34.29	64.29	72.46	67.83
Lunalim	33.44	63.44	66.31	64.56

Apollo	35.17	65.17	71.11	70.0
Atlas	15.56	45.56	49.05	50.5
Delux	31.43	61.43	68.92	66.5
UC157	29.0	59.0	60.8	63.42
Grande	27.94	57.94	65.0	62.4
WB212	33.41	63.4	70.8	63.76
WB230	25.75	53.97	68.7	61.7
WB231	23.98	53.7	64.56	60.49
Purple passion	20.9	50.9	55.2	54.6

All varieties were susceptible to stem blight. In May, 25 months after planting, the disease began to appear at a low rate ranging from 15.6% to 35.17%, the lowest in the Atlas and Purple passion varieties and the highest in Apollo. However, in August and September after the increase in temperature and heavy rains, the disease rate increased sharply. The disease rate reached its highest in September, the highest rate was 72.46% in Starlim, followed by Apollo with 71.11%. The disease rate is lowest in the Atlas variety at 49.0% and in Purple passion at 55.2%. By October, when the temperature decreased, the disease rate tended to decrease slightly.

3.5. Asparagus yield productivity and yield component of imported asparagus varieties

The number of spears in the spring season, of 2022 tended to be higher than that in the autumn season of 2022. In both seasons, the Atlas variety had the highest number of spears and was significantly different from the other varieties (Table 6).

The average weight of spears was the highest in the Grande variety in both seasons, significantly different from the rest of the varieties, followed by the Atlas variety. In spring 2022, the average weight of spears of the varieties Apollo, Lunalim, and WB231 was not different from Atlas and was all high. In the autumn season of 2022, the average weight of spears of the Purple passion, Atlas, and UC157 were not significantly different.

Table 6. Asparagus yield and yield component of imported asparagus varieties

Variety	Number of spears cluster^{-1}		Average weight of spears (g spear^{-1})		Individual yield (g plant^{-1})		Yield productivity (quintal ha^{-1})	
	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022
K767	7.5b	4.9bc	18.5de	9.6cde	137.1c	47.0bc	32.4bc	7.0cd
Starlim	6.8bcd	4.7bc	13.3h	8.8ef	90.6e	41.2c	27.1de	6.2d
Lunalim	6.8bcd	4.4bc	19.8bcd	9.2def	134.2c	40.5c	29.2cd	6.7cd
Apollo	6.5cde	4.2bc	20.2bc	8.8ef	133.9c	36.6c	29.4cd	7.0cd
Atlas	8.6a	6.4a	21.0b	10.9b	180.3a	69.5a	37,5a	9.8a
Delux	6.7bcde	4.2bc	15.9g	8.6f	106.3de	36.1c	28.5de	6.4d
UC157	7.2bc	5.5ab	18.6de	9.8bc	131.4c	53.9b	34.2ab	7.0cd
Grande	7.0bcd	5.4ab	22.4a	12.4a	157.9b	67.3a	34.0ab	8.7b
WB212	5.7ef	4.1c	19.2cde	9.3def	109.9d	37.9c	24.4e	6.4d
WB230	6.1def	4.8bc	18.2ef	9.2def	111.6d	44.2bc	26.3de	6.9cd
WB231	6.8bcd	5.1abc	19.7bcd	9.5def	133.1c	48.3bc	26.2de	7.1cd
Purple passion	5.3f	4.2bc	17.0fg	10.5bc	89.7e	43.7bc	25.2e	7.6c

Note: Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

The individual yield of the spring season of 2022 tended to be higher than that of the autumn season of 2022. In both seasons, the individual yield of the Atlas variety had the highest value, which was significantly different from the other varieties, followed by the Grande variety.

The yield productivity of the spring season of 2022 was higher than that of the autumn season of 2022. The yield productivity of the Atlas variety had the highest value in both seasons and was significantly different from the other varieties; the lowest ones were the WB212 and Starlim varieties. In the spring season of 2022, the yield productivity of UC157 and Grande was not significantly different from that of the Atlas variety. In the autumn season of 2022, the yield productivity of Atlas reached the highest value, followed by Grande, Purple Passion, UC157, and K767 (Table 6).

3.6. Quality characteristics of imported asparagus varieties

The Brix of spears of imported asparagus varieties was measured in the autumn season of 2022; only UC157 and WB231 varieties had the highest Brix of spears, followed by K767, Atlas, and Purple Passion (Table 7).

The vitamin C content of asparagus varieties fluctuated significantly in both seasons. In both seasons, vitamin C content was highest in the Purple passion variety, followed by the Atlas variety. In the spring season of 2022, the vitamin C content of asparagus varieties ranged from 9.49 to 19.25 mg $100g^{-1}$. In the autumn season of 2022, vitamin C content fluctuated more than in the spring season from 8.3 to 20.5 mg $100g^{-1}$ (Table 7).

Table 7. Quality characteristics of imported asparagus varieties

Variety	Brix of spear	Vitamin C content (mg $100g^{-1}$)		Total sugar content (%)		Fiber content (%)
	Autumn 2022	Spring 2022	Autumn 2022	Spring 2022	Autumn 2022	Autumn 2022
K767	6.7b	11.05h	9.66f	1.96a	1.99de	2.14e
Starlim	5.5e	11.64g	14.94b	1.80b	2.09bcde	2.4a
Lunalim	6.3c	9.74i	10.6e	1.60g	1.85e	2.1f
Apollo	6.3c	15.71cd	12.65cd	1.64ef	2.21bcd	2.3c
Atlas	6.5bc	16.32b	14.9b	1.64ef	2.37b	1.59i
Delux	5.9d	14.79e	12.4d	1.67de	1.99de	2.34b
UC157	7.4a	9.49j	14.89b	1.60g	2.04cde	2.35b
Grande	6.3c	12.08f	8.3g	1.66h	2.30bcd	2.11f
WB212	5.5e	15.87c	13.4c	1.75c	2.31bcd	1.94h
WB230	7.3a	9.97i	15.3b	1.68d	2.35bc	2.3c
WB231	6.1cd	15.56d	10.1ef	1.48i	1.99de	2.02g
Purple passion	6.4bc	19.25a	20.5a	1.67de	3.15a	2.16d

Note: Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

The NO_3^-content threshold for asparagus is 200 mg kg^{-1} (WHO standard), in the autumn season of 2022, all varieties of asparagus had NO_3^-content below the permissible threshold and were safe for human consumption.

The total sugar content of the autumn season of 2022 tended to be higher than that of the Spring season of 2022. In the Spring season 2022, the K767 variety had the highest total sugar content, followed by Starlim, and was significantly different from the other varieties. In the autumn season of 2022, the Purple passion variety had the highest total sugar content and was significantly different from the other varieties, followed by Atlas, WB230, WB212, Grande, Apollo, and Starlim (Table 7).

The fiber content of the asparagus varieties in the autumn season of 2022 ranged from 1.59 to 2.4%. The Atlas variety had the lowest fiber content and was significantly different from the other varieties.

3.7. Relationship between Asparagus yield productivity and root Brix

The relationship between asparagus yield productivity and root Brix were positive and significant in both the spring and autumn seasons 2022 (Figure 3).

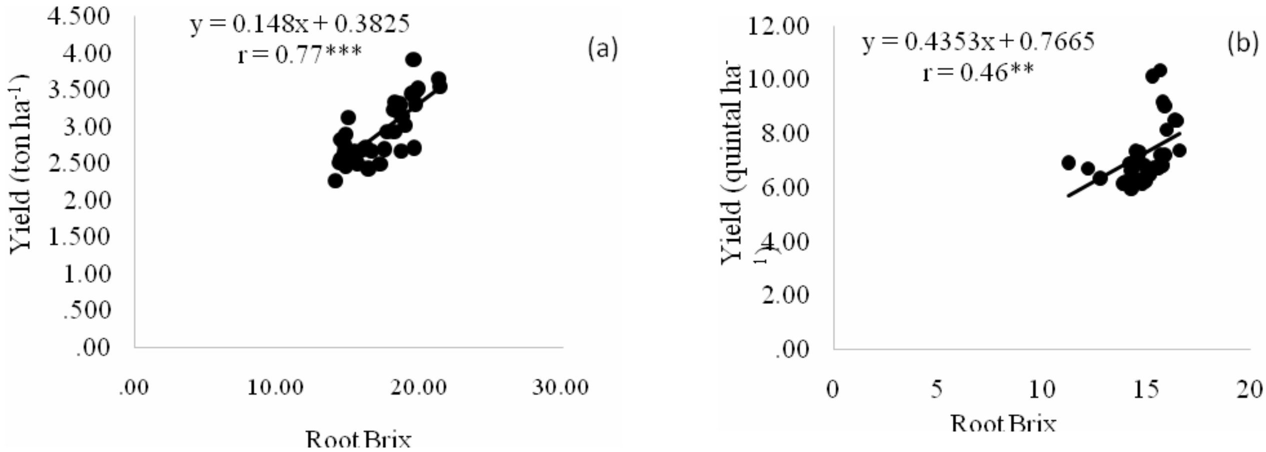

Figure 3. The relationship between Asparagus yield productivity and root Brix in the spring season 2022 (a) and autumn season 2022 (b). **, *, significant at (P)< 0.01 and (P)<0.001, respectively**

4. DISCUSSION

The Northern of Vietnam belongs to the tropical and subtropical regions, reported the high temperature and humidity, and adaptability of asparagus varieties can provide a good yield. However, stem blight has been a constraint to extending asparagus growing areas in the Red River Delta in Vietnam as well as other regions in Vietnam and other Asian and Pacific countries such as Japan, China, Myanmar, Australia, and New Zealand [15]. Until now, no resistant asparagus variety has been developed. There were no effective chemical synthetics to control disease [16]. Therefore, it is necessary to have integrated management the asparagus under the field to control the disease, such as plant density, nutrition management, and increase biodiversity, especially growing under plastic tunnels or mulch to reduce the possibility of *Phomopsis asparagi* spores reaching the foliage. In this study, Atlas could be a good variety to resist *Phomopsis asparagi* infection because of its lower infection rate (Table 5).

Among the varieties evaluated, the Atlas variety had the highest yield productivity and yield components (Table 6) because the Atlas variety had the highest root Brix, highest root CHO (Table 3), and highest dry matter weight (Table 4), the rate of diseases caused by *Phormopsis asparagi* was low (Table 5). The research results of Nguyen *et al.* (2020) [9] also pointed out that Atlas had a high spear yield, the ratio of spear grades 1,2 was over 30%, and the economic variety was suitable for South Central Vietnam. Moreover, the cultivar of Atlas had a high tolerance to soil-borne disease caused by *Fusarium* sp. and leaf rust caused by *Cercospora* sp. Atlas can adapt to the tropical medium environment, which is constrained by high temperature and humidity [17]. Besides, the reasons why the number and weight of asparagus spears yield higher than the others in this study, and that's why Atlas is well adapted to the hot, humid climate of the Red River Delta, Vietnam.

Besides the advantage of the thickness of the spears, purple asparagus has been expected to contain more antioxidant content than green asparagus. Purple passion has been approved since it contains the novel anthocyanin A1 cyanidin 3-[300-(O-b-D-glucopyranosyl) - 600 - (O - a - L - rhamnopyranosyl) - O - b - D - glucopyran - oside] and the anthocyanin A2 cyanidin 3-rutinoside with high antioxidant activities [18]. In addition, the high rutin content, high chl a and b, and total carotenoid content were found in the 'Purple passion' variety [21]. That is the reason why consumers can pay a higher price for the products and can compensate for the low yield of this Purple passion variety.

Asparagus yields are linked to the availability of soluble carbohydrates (CHO) in the storage root system, which is considered a key factor in asparagus productivity. Asparagus yield and plant growth are highly dependent on the availability of soluble carbohydrates (CHO) in the storage root system [11]. Ultimately, root CHO levels are considered to be a key factor determining asparagus yield performance, which was officially recognized by the AspireNZ decision support system of Wilson *et al.* (2002) [20], which provided growers with an interpretation guide of root CHO content to facilitate better crop management decisions. There is significant variation in asparagus storage root CHO levels between plants depending on the size of the root system [11]. In this study, root Brix was measured before harvesting and decision the time of harvest asparagus and using the root Brix data to convert to root CHO. There was a positive and significant correlation between root Brix and yield productivity of 12 asparagus varieties in both seasons (spring and autumn seasons 2022), this finding agreed with the finding of Maˇskov´a *et al.* (2023) [21] that asparagus yields are linked to the availability of soluble carbohydrates (CHO) in the storage root system via root Brix, which is considered a key factor in asparagus productivity.

5. CONCLUSION

The growth, physiological, yield productivity, and yield components of asparagus varieties in the spring season of 2022 tended to be higher than those of the autumn season of 2022. Sterm blight due to Phormosis asparagi infection is very prevalent in September in the autumn season of 2022. Atlas is the most promising asparagus variety that can be grown commercially because of the advantages of having the largest spear diameter, root Brix, and CHO of the roots, and the highest dry matter weight, yield productivity, and yield components as compared with other varieties. Besides, quality characteristics such as Brix of spears, vitamin C content of spears were high, and fiber content of spears was the lowest. Some promising varieties of asparagus are Grande and Purple Passion which are also promising varieties after Atlas. In addition, the Purple passion variety is special because it has the highest vitamin C content of spears, the highest total sugar content of spears in the autumn season of 2022, and has more anthocyanin content in spears compared to the other varieties. From these results, Purple passion is also a promising variety for commercial production, although the yield is not high.

There was a positive and significant correlation between asparagus yield productivity and root Brix in both seasons in 2022. The results suggestst that the root Brix of asparagus could be the target trait for choosing varieties for production.

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EFFECTS OF GRAFTING ROOTSTOCKS, SEASONS AND METHODS ON SPROUTING RATE AND SHOOT GROWTH OF SUGAR APPLE LINE Na16 (*Annona squamosa*) IN THE NURSERY STAGE

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ABSTRACT

The sugar apple line Na16 (*Annona squamosa*) selected from production by the Fruit and Vegetable Research Institute had a variety of valuable attributes. In order to improve the efficiency of the sugar apple line Na16 production, the study on the influence of grafting rootstocks, seasons and methods to the sprouting rate and growth of grafted plants in the nursery stage was conducted from January 2020 to December 2021. The research results indicated that suitable sugar apple rootstocks included the two varieties Na Dai and Na Bo, and grafting in Ha Noi from January 20 to January 25 found to be most favorable. The sprouting percentage of Na16 plants grafted by side graftingduring this period reached over 90% and the success rate ranged from 73.61 to 75.28%.

Keywords: *Sugar apple line Na16, grafting method, grafting season, rootstock, sprouting rate.*

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1. INTRODUCTION

The sugar apple line Na16 was selected in Chi Lang district, Lang Son province by the Fruit and Vegetable Research Institute. Originated in China and introduced through cross-border into Vietnam, the line Na16 had a variety of valuable attributes such asvigorous growth, large fruit weight (500 - 800 g/fruit), high yield, small number of seeds (<30 seed/fruit), fragrant, firm and well-structuredfleshas well as a sweet and delicate flavour.

The reproduction of sugar apple take place both sexually (from seeds) and asexually (by grafting) [1]. Vegetative propagation of sugar apple is commonly implemented with the help of patch budding, side grafting and cleft grafting [2], [3]. Besides, most species of the genus Annona can be propagated from stem and root cuttings [4]. In Vietnam, new cultivation of traditional sugar apple varieties mainly utilizes seedlings obtained from seeds (i.e. Na Dai, Na Bo). However, propagation of new varieties (such as Na Hoang Hau, Na Dua...) mainly employ vegetative methods, including grafting (i.e. side grafting and inarch grafting) and patch budding. However, each variety varies in preferable rootstocks, seasons and methods for grafting [5], [6]. There are currently limited studies on rapid multiplication and expanding cultivation area of the sugar apple line Na16.

In order to develop a propagation protocol on the sugar apple line Na16 to extend its production, the influences of factors includingrootstocks, grafting seasons and methods on sprouting rate and growth of grafted plants were studied.

2. MATERIALS AND METHODS

2.1. Materials

- Rootstocks for grafting: Rootstock plants were germinated from seeds of Na Dai, Na Bo and Na Thai varieties.

- Scions for grafting: Sugar apple line Na16 obtained from mother plants of 3 - 4 years old.

- Fertilizers and other materials: Microbiological fertilizers, superphosphate (16% P_2O_5), urea (46% N), potassium chloride (60% K_2O), pesticides, foliar fertilizers, etc. and garden tools (saws, secateurs, grafting knives, grafting strips, etc.)

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2.2. Methods

2.2.1. Experiment design

- Experiment 1: Influence of rootstock varieties on sprouting rate and shoot growth of Na16 grafted lants in nursery stage.

- The experiment 1 was conducted with 3 treatments:

+ Treatment 1 (CT1): Grafting on the rootstocks of Na Dai variety.

+ Treatment 2 (CT2): Grafting on the rootstocks of Na Bo variety.

+ Treatment 3 (CT3): Grafting on the rootstocks of Na Thai variety.

The experiment was conducted in a Randomised Complete Block Design (RCBD) with 3 replications and 120 grafted plants in each treatment of each replication. The grafting was carried out from January 20, 2020 to January 25, 2021. Apart from the experimental factor, which was the rootstocks, other non-experimental factors were maintained identical in the experiment.

- Experiment 2: Influence of grafting seasons and methods on sprouting rate and shoot growth of Na16 grafted plants in nursery stage

- The experiment 2 was designed with two experimental factors:

+ Factor 1 (P factor: Methods of grafting) including 2 grafting methods of side grafting (P1) and patch budding (P2).

+ Factor 2: (T factor: Seasons of grafting) including 3 grafting seasons in January (T1), in June (T2), and September (T3).

- The specific grafting time in the years were as follows:

+ In January: Grafting was conducted on January 20, 2020 and January 25, 2021.

+ In June: Grafting was conducted on June 16, 2020 and June 18, 2021.

+ In September: Grafting was conducted on September 18, 2020 and September 16, 2021.

- The experiment 2 consisted of 6 treatments: Treatment 1 (T1P1); treatment 2 (T2P1); treatment 3 (T3P1); treatment 4 (T1P2); treatment 5 (T2P2); and treatment 6 (T3P2).

- The experiment was laid out in a RCBD with 3 replications, with 120 grafted plants in each treatment of each replication. Apart from the experimental factors, which were grafting seasons and methods, other non-experimental factors were maintained identical.

2.2.2. Experimental indicators and methods of monitoring and evaluation

- Sprouting rate (%) = Number of sprouted grafts forming new shoots/Total number of grafted plants*100.

+ Shoot length (cm): Measured from the shoot base to the shoot tip.

+ Shoot diameter (cm): Measured with a caliper at 2 cm from the shoot base.

- Growth ability of grafted plants: The length and diameter of new shoots forming on grafted plants were monitored, with 10 plants per treatment and with 3 replications, at 60 days after grafting.

- Grafting success rate (%): Number of plants meeting the standards for transplanting/Total number of grafted plants*100. The standards of success grafts were as follows: Plant height from potting medium surface to the plant tip should be over 40 cm; the length of the new shoot from the scion should be over 30 cm.

2.2.3. Data processing

Research data were collected with the biological statistical method and processed using Excel and CropSTAT 7.2 softwares.

2.3. Time and location

The study was conductedat the Fruit and Vegetable Research Institute (FAVRI), Ha Noi, Vietnamin 2 years, from January 2020 to December 2021.

3. RESULTS AND DISCUSSION

3.1. Influence of rootstock varieties on sprouting rate and shoot growth of Na16 grafted plants in nursery stage

3.1.1. Influence of rootstock varieties on sprouting rate of Na16 grafted plants

In the production of fruit plant seedlings in general and sugar apple seedlings in particular, the proportion of grafted plants forming new shoots is always an important criterion that determines the efficiency of propagation. There are many factors affecting the sprouting rate after grafting such asscion quality, grafting technique, weather condition during and after grafting, etc. Once the above factors were the same among all treatments, the sprouting rate would be mainly affected by the compatibility between the rootstocks and the scions.

The following table describes results of monitoring the influence of rootstock varieties on the sprouting rate of Na16 line.

The data indicated that with the weather conditions in Ha Noi, grafting combination of Na16 scions on Na Thai rootstocks resulted in significantly lower rates of shoot formation (at 86.39% in 2020 and 85.28% in 2021) than those of the other rootstocks. The sprouting rate of Na16 grafted on Na Dai and Na Bo rootstocks were relatively higher, ranging from 89.72% to 91.11%, with statistically insignificant difference in the rates of these two rootstocks.

Table 1. Influence of rootstock varieties on sprouting rate of Na16 grafted plants

Treatments	Sprouting rate (2020)		Sprouting rate (2021)	
	(%)	Arcsin	(%)	Arcsin
CT1 (Na Dai rootstocks)	90.83	72.39	90.56	72.24
CT2 (Na Bo rootstocks)	91.11	72.79	89.72	71.42
CT3 (Na Thai rootstocks)	86.39	68.36	85.28	66.86
$LSD_{0.05}$		*2.69*		*3.57*
CV (%)		*1.7*		*22*

Therefore, among the rootstocks used for grafting of Na16 scions in the experiment, the treatment combination with Na Thai variety showed the lowest sprouting rates, while grafting with Na Dai and Na Bo varieties had relatively higher rates. There was insignificant difference in sprouting rates of grafting with Na Dai and Na Bo varieties.

3.1.2. Influence of rootstock varieties on shoot growth of Na16 grafted plants in nursery stage

The growth ability of the grafted plants determines the quality of the young plants for transplanting, reflected in the length and diameter of new shoots formed. The table 2 below illustrates the data of the shoot growth ability of Na16 grafted on different rootstocks monitored at 60 days after grafting.

The data presented in table 2 indicated that grafting Na16 scions on Na Thai rootstocks not only resulted in low sprouting rates, but the size of new shoots were also significantly smaller than those of the grafting combination on Na Dai and Na Bo rootstocks. The combination of Na16 and Na Thai recordedrelatively smaller shoot length and diameter, ranging in 36.70 - 38.83 cm and 0.61 - 0.62 cm.Meanwhile, the later combinations produced better new shoots. The length and diameter were insignificantly different, varying from 46.70 to 50.42 cm, and from 0.73 to 0.76 cm.

Table 2. New shoot growth of Na16 grafted plants at 60 days after grafting

Treatments	Year 2020		Year 2021	
	Shoot length (cm)	Shoot diameter (cm)	Shoot length (cm)	Shoot diameter (cm)
CT1	48.74	0.77	50.42	0.76
CT2	46.70	0.73	49.64	0.74
CT3	36.70	0.61	38.83	0.62
$LSD_{0.05}$	*6.27*	*0.04*	*2.78*	*0.03*
CV (%)	*6.3*	*2.7*	*2.6*	*1.9*

According to the formation of new shoots from the scions, the rootstocks of Na Dai and Na Bo varieties revealed to be suitable for the scions Na16.

3.1.3. Influence of rootstock varieties on the grafting success rate of Na16

The grafting success rate of Na16 scions on different rootstocks are described in the table 3.

Table 3. Influence of rootstock varieties on the grafting success rate of Na16

Treatments	Grafting success rate (2020)		Grafting success rate (2021)	
	(%)	Arcsin	(%)	Arcsin
CT1	70.83	57.33	70.28	56.98
CT2	69.44	56.45	68.33	55.82
CT3	47.50	43.56	45.28	42.27
$LSD_{0.05}$		*1.39*		*8.10*
CV (%)		*1.2*		*6.9*

The data in the table 3 showed that: among the tested rootstocks, the combination with Na Thai rootstocks led to the lowest grafting success rate for transplanting, with 47.5% in 2020 and 45.28% in 2021. Similar to the criteria of new shoots formation, the grafting success rate in the treatment 1 and 2 (CT1 and CT2) were statistically insignificantly different, ranging of 69.44 - 70.83% in 2020 and 68.33 to 70.28% in 2021.

Therefore, the combination of the Na16 scions with rootstocks of Na Dai and Na Bo varieties in the experiment resulted in better sprouting and success rates than those of the combination with Na Thai rootstocks.

3.2. Influence of grafting seasons and methods on sprouting rate and shoot growth of Na16 grafted plants in nursery stage

3.2.1. Influence of grafting seasons and methods on sprouting rate of Na16 grafted plants

Table 4 depicts monitoring data of the influence of grafting seasons and methods on sprouting rate of the line Na16 at the Fruit and Vegetable Research Institutein 2020 and 2021.

Table 4. Influence of grafting seasons and methods on sprouting rate of Na16 grafted plants

Factors	Sprouting rate(2020)		Sprouting rate (2021)	
	(%)	Arcsin	(%)	Arcsin
Grafting seasons - T				
T1 - January	85.33	68.16	85.00	67.62
T2 - June	82.50	65.43	80.56	64.07
T3 - September	78.61	62.56	76.53	61.17
$LSD_{0.05}$		*2.19*		*1.86*
Grafting methods – P				
Side grafting- P1	86.48	68.59	86.20	68.40
Patch budding - P2	78.14	62.18	75.19	60.18
$LSD_{0.05}$		*1.79*		*1.51*
Interaction effect of grafting seasons and methods				
T1P1	90.28	71.88	90.83	72.39
T2P1	86.67	68.59	86.11	68.14
T3P1	82.50	65.30	81.67	64.66
T1P2	81.39	64.45	79.17	62.86
T2P2	78.33	62.27	75.00	60.00
T3P2	74.72	59.83	71.39	57.67
$LSD_{0.05}$		*3.10*		*2.62*
CV (%)		*2.4*		*2.0*

The data in table 6 indicated that in the condition of Ha Noi in 2020 - 2021, grafting seasons and methods had a significant impact on the sprouting rate of Na16 grafted plants in the nursery stage.

- *Influence of grafting seasons:* Different grafting seasons in January (T1), June (T2) and September (T3) resulted in different effects on the rate of shoot formation of Na16 in the nursery stage. In 2020, the rate of new shoot formation in the treatment T1 (85%) was remarkably higher than those of the treatments T2 and T3, ranging from 80.56 to 82.5% and 76.53 to 78.61%, respectively; the same trend was also reported in 2021. Therefore, it can be concluded that: Grafting of the line Na16 in January resulted in the highest sprouting rate followed by that in June, and the lowest rate was in September.

Many studies on grafting seasons on other fruit trees demonstrated that the suitable season for grafting in northern provinces is autumn season (August to October) [7]. However, in regard to sugar apple trees, autumn grafting (in September in our experiment) might lead to the lowest sprouting rate. This is possibly due to their characteristics as deciduous trees. The vegetative growth begins to retard in autumn time, resulting in the formation of new shoots slowing down [8]. Meanwhile, January is the time of the year when both the rootstock and the scion varieties areswitching fromthe dormant stage to the vegetative growth stage, leading to higher sprouting rates. The findings suggest a need for further fundamental studies to serve asscientific evidence and reference for propagation of sugar apple in Northern Vietnam.

- *Influence of grafting methods:* Analysis results revealed that in both experimental years (2020 and 2021), there was a clear difference in the sprouting rate of Na16 between the two grafting methods. In particular, the side grafting method gave significantly higher rates (reaching over 86% in both years) than the patch budding method (at 78.14% in 2020 and 75.19% in 2021). Thus, it can be initially concluded that the side grafting method was relatively suitable for grafting of the line Na16.

- *Effect of interaction between grafting seasons and methods:* The interaction between grafting seasons and methods was clearly proved in the rates of new shoot formation of Na16 grafted plants, which were dissimilar to the influence of individual factors. In 2020, different grafting methods (P1 and P2) resulted in varying sprouting rates in only two grafting seasons T1 and T3; no variation was recorded in grafting season T2. Meanwhile, the individual influence of grafting seasons (T1, T2, T3) on sprouting rate varied significantly in the experiment. In 2021, the same figure was seen in treatments utilizing side grafting technique (P1); whereas, the influence of patch budding treatments was clearly revealed since the sprouting rate in T2P2 treatment was different from that in T1P2 treatment but not that in T3P2.

From the above analysis, it can be concluded that among the experimental factors, the most suitable condition for grafting of the line Na16 was the side grafting method in January.

3.2.2. Influence of grafting seasons and methods on shoot growth of Na16 grafted plants in nursery stage

The growth ability of grafted plants determines their quality for transplanting, which is reflected in the length and diameter of the new shoots. Monitoring the main characteristics of the grafted plants in different grafting seasons and methods at 60 days after grafting revealed the results shown in table 5.

Table 5. Main characteristics of Na16 grafted plants at 60 days after grafting

Factors	Year 2020		Year 2021	
	Shoot length (cm)	Shoot diameter (cm)	Shoot length (cm)	Shoot diameter (cm)
Grafting seasons				
T1 - January	45.49	0.70	49.44	0.70
T2 - June	43.54	0.67	44.80	0.65
T3 - September	39.57	0.60	40.56	0.61
$LSD_{0.05}$	*1.29*	*0.03*	*3.08*	*0.03*
Grafting methods				
Side grafting- P1	46.25	0.73	47.87	0.75
Patch budding - P2	39.47	0.58	42.00	0.56
$LSD_{0.05}$	*1.05*	*0.02*	*2.52*	*0.02*
Interaction effect of grafting seasons and methods				
T1P1	50.48	0.79	51.59	0.80
T2P1	46.18	0.75	48.93	0.75
T3P1	42.10	0.65	43.08	0.69
T1P2	40.50	0.60	47.30	0,61
T2P2	40.89	0.57	40.67	0,55
T3P2	37.03	0.54	38.04	0,52
$LSD_{0.05}$	*1.82*	*0.03*	*4.36*	*0,03*
CV (%)	*2.1*	*3.0*	*4.9*	*3,2*

The data in table 5 indicated the significant effect of grafting seasons and methods on the growth of the line Na16 in nursery stage in the year 2020 and 2021.

- *Influence of grafting seasons:* The length and diameter of new shoots reached the highest values in the grafting season of January, followed by that of June grafting. The lowest values were recorded in

September grafting. This may be due to the fact that the young Na16 plants had favorable weather conditions for their vegetative growth after grafting in January. In regard to young plants of September grafting, the newly-formed shoots might encounter unsuitable temperature and humidity conditions for vegetative growth.This was also the time that sugar apple trees were about to enter the dormant phase.

- *Influence of grafting methods:* Different grafting methods had various effects on the growth of Na16 grafted plants. The treatment P1 of side grafting obtained significantly larger shoot size than that of the patch budding treatment (P2).

- *Effect of interaction between grafting seasons and methods:* In general, the influence of interaction between grafting seasons and methods on the length and diameter of new shoots was unclear in both years 2020 and 2021. Such vegetative growth of new shoot of Na16 grafted plants was mostly similar to that in consideration of each factor separately. Hence, there was a statistically significant difference in the length and diameter of new shoots on Na16 grafted plants in different grafting seasons and with different methods. The highest size of new shoots were obtained in January grafting treatment.

From the above results, it can be concluded that the most suitable treatment for the growth of Na16 grafted plants was T1P1 (side grafting in January), followed by T2P1 treatment (side grafting in June) and T3P1 treatment (side grafting in September).

3.2.3. Influence of grafting seasons and methods on the grafting success rate of Na16

Influence of the grafting seasons and methods on the grafting success rate for transplanting is described in table 6.

Table 6. Influence of grafting seasons and methods on the grafting success rate of Na16

Factors	Grafting success rate (2020)		Grafting success rate (2021)	
	(%)	Arcsin	(%)	Arcsin
Grafting seasons - T				
T1 - January	62.36	52.36	61.81	52.12
T2 - June	53.75	47.27	55.56	48.37
T3 - September	50.00	45.00	51.39	45.85
$LSD_{0.05}$		*1.93*		*1.68*
Grafting methods – P				
Side grafting- P1	69.26	56.38	70.74	57.33
Patch budding - P2	41.48	40.05	41.76	40.23
$LSD_{0.05}$		*1.57*		*1.37*
Interaction effect of grafting seasons and methods				
T1P1	73.61	59.09	75.28	60.19
T2P1	70.28	56.27	71.39	57.67
T3P1	63.89	53.07	65.56	54.13
T1P2	51.11	45.64	48.33	44.04
T2P2	37.22	37.59	39.72	39.07
T3P2	36.11	36.93	37.22	37.58
$LSD_{0.05}$		*2.72*		*2.38*
CV (%)		*2.8*		*2.4*

The results in table 6 exhibited that there was a clear difference in the grafting success rate of Na16 due to different grafting seasons and methods in 2020 and 2021:

- *Influence of grafting seasons:* Grafting in January resulted in the highest grafting success rate for transplanting, followed by June grafting and September grafting.

- *Influence of grafting methods:* There was a significant difference in treatments of different grafting methods. The grafting success rate in side grafting treatment (P1) was remarkably higher than that in treatment P2 (patch budding).

- *Interaction effect of grafting seasons and methods:* Influence of grafting seasons and methods in combination on the grafting success rate for transplanting was relatively similar to that in consideration of each individual factor. The highest rate of young plants available for transplanting was seen in treatment T1P1 (side grafting in January), followed by that in treatment T2P1 (side grafting in June) and lastlythat in treatment T3P1 (side grafting in September).

In summary,grafting of Na16 by side grafting in January led to the highest grafting success rate for transplanting, while the lowest was that of patch budding in September.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

- Among the varieties in the research, Na Dai and Na Bo rootstocks were the most suitable for the scions of Na16.

- In the experiment of grafting seasons and methods, side grafting in January was the most favourable for the growth of the line Na16, with the sprouting rate reaching over 90% and the grafting success rate ranging from 73.61 to 75.28%.

4.2. Recommendation

The research findings are suggested to be included in cultivation protocols on the sugar apple line Na16 and applied in practice in the North of Vietnam.

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RESULTS ON BREEDING AND SELECTING OF NEW PROMISING STRAWBERRIES FOR HIGHLANDS IN VIETNAM

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ABSTRACT

Research on breeding and selecting promising strawberry clones has been carried out at the Potato, Vegetable and Flower Research Center in Da Lat city, Lam Dong province and Bac Ha district, Lao Cai province since 2018. The research materials were imported from Korea, Japan and - local varieties in Vietnam. Applying sexual hybridization method to create hybrid combinations, evaluation and selection hybrid individuals combined with asexual propagation through tissue culture method was used in this study. As a result, 50 hybrid combinations were created through the research with 10.800 seeds. Six promising clones including PS1.07, PS7.01, PS8.07, PS8.10, PS8.14, PS17.04 were selected for trials that applying high technology in the net houses in Da Lat city, Lam Dong province and Bac Ha district, Lao Cai province. They are considered with high yield potential (27.8-32.6 tons/ha/year in Da Lat city and 12.7-13.2 in Bac Ha district), first classified fruit (73.6-82.1%), fruit sweetness (Total soluble solids(^{0}Brix):11.0 - 12.6%), good taste, very fragrant and firmness fruit; tolerated to some main diseases such as powdery mildew (*Sphaerotheca macularis*), Anthracnose (*Colletotrichum fragariae*) and Angular leaf spot (*Xanthomonas fragariae).* Among of 6 promising tested clones, PS8.10 demonstrated to be a remarkable promising clone for high yield (32.6 tons/ha/year in Da Lat city and 13.2 tons/ha/year in Bac Ha district) that it was highly accepted by farmers in productions of Lam Dong and Lao Cai provinces.

Keywords: *Strawberry, promising clones, Da Lat - Lam Dong, Bac Ha - Lao Cai.*

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1. INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) is a commonly grown hybrid species and cultivated worldwide for their fruits [1] due to the fact that strawberry is one of the most important berry fruits and is accepted by a large number of consumers around the world [2]. Strawberries have countless health benefits as well, such as preventing some certain cancers, enhancing cardiovascular health, boosting insulin sensitivity and controlling a healthy immune system [3, 4]. In 2019, world production of strawberries was 9,125,913 ton, led by China with 3,801,865 ton and the United States with 1,420,570 ton. These two countries together got approximately 57% of the World's total in 2019.

Breeding and selecting of strawberries were strongly invested and developed in most advanced countries such as the United States, Canada, French, Netherlands, Spain, etc. In the 15th century, breeding started with selection and cultivation of European strawberry species in western Europe. While in 1966, a similar discovery and cultivation just occurred in Chile [5]. However, the first actual actions were carried out in the United Kingdom, the United States, France, Germany in the 19th century [6]. The program of breeding strawberries was mainly directed towards enhancing agronomic performance, resulting in varieties which produced high yields of large red and firm fruits, but fruit aroma was the major quality trait that had a strong impact on consumers [7, 8].

So far, there have been hundreds of strawberry varieties created by public agencies and private companies. The breeding of strawberries started

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with traditional methods and moved on to molecular breeding and genetic engineering in the 20th century to supply to the world's demand for fruits by overcoming adverse conditions and diseases. The back-cross method was also applied in cases to transfer essential genes, especially for transfering resistant genes or adaptive traits from wild species into *F. x ananassa*. The aphid resistance of *F. chiloensis* neutrally reacted to the photoperiod of *F. virginiana* spp. glauca was converted to *F. x ananassa* by this method [9, 10, 11].

In Vietnam, strawberries were mainly carried and grown in Da Lat city, Lam Dong province before 1970. In 1965, the commercial area for strawberries in Da Lat city was estimated to be around 10 hectares (ha) [12]. Up to now, the commodity strawberry area in Lam Dong province is about 200 ha. Lao Cai province is also a potential location with 30 ha for the development of strawberry. Strawberry breeding research in Vietnam is a newly developed field so it currently stops at collecting, importing, and selecting new varieties. From 2003 to 2011, Potato, Vegetable and Flower Research Center imported some varieties from Taiwan and the United States to conduct the research and evaluate, select, crossbreed to create new varieties under Da Lat city climatic conditions. As a result, two varieties Angelis (My Da) and Camarosa (Langbiang 2) were successfully selected during this period. They are the main varieties to open production field in Da Lat city, Lam Dong province, in which the Angelis has accounted predominantly for nearly 60% of the growing area, with an average yield of 13-14 tons/ha/year. During the past years, some organizations and individual enterprises had imported several varieties such as Albion, Monterey, New Zealand, Go Ha, Ha Na, Akihime, White Jewel from the United States, New Zealand, Korea and Japan for experiments in the net house conditions in Lam Dong province. The results showed that the New Zealand (Brix level at 7.5 - 8.7%) was the most suitable variety with high potential yield with red color, beautiful appearance, and slight firmness [13]. Whereas the cultivars from Korea and Japan had good quality but soft firmness and high sensitivity to Powdery mildew. These varieties are seriously degenerating leading to low productivity and quality. Therefore, new varieties need to be created to meet the demand for strawberry production.

2. MATERIALS AND METHODS

2.1. Time and location research

The researches were conducted from January 2018 to May 2021 in Da Lat city, Lam Dong province and Bac Ha district, Lao Cai province:

+ Cross-breeding, harvesting and sowing intervarietal crosses: January 2018 to April 2018, carried out in Da Lat city, Lam Dong province;

+ Growing cycle 1 (C1): May 2018 to December 2018, carried out Da Lat city, Lam Dong province;

+ Growing cycle 2 (C2): May 2019 to the end of May 2020, carried out in Da Lat city, Lam Dong province;

+ Experiment design: May 2020 to the end of May 2021, carried out in Da Lat city, Lam Dong province and Bac Ha district, Lao Cai province;

2.2. Materials

Cross-breeding: 18 varieties were introduced from the United States, New Zealand, Korea, Japan, and other local varieties being produced in Da Lat city, Lam Dong province, in which mainly focused on varieties with high yieldsranging from 20-30 tons/ha/year and Brix level ranging from 9-10%. Moreover, these varieties were resistant to pests and diseases.

Selection cycle 1 (C1): 10.800 seeds of 50 intervarietal crosses were created.

Selection cycle 2 (C2): 18 promising clones in cycle 1 (C1): PS1.01, PS1.05, PS1.06, PS1.07, PS3.01, PS7.01, PS7.02, PS8.01, PS8.02, PS8.03, PS8.07, PS8.09, PS8.10, PS8.14, PS17.01, PS17.02, PS17.03, and PS17.04.

Evaluation and chosen best promising clones:

+ 06 promising clones in cycle 2 (C2): PS1.07, PS7.01, PS8.07, PS8.10, PS8.14, and PS17.04.

+ 06 promising clones in cycle 3 (C3): PS1.07, PS7.01, PS8.07, PS8.10, PS8.14, and PS17.04 for trials on Da Lat city and Bac Ha district.

2.3. Methods

2.3.1. Cross-breeding method and selecting new clones

The program of breeding strawberry is recently applied to acquire new varieties improved for specific agronomic (yield and size), qualitative (firmness, sugars content and acidity) and sensorial (color and aroma) characteristics, all combined to level up the disease resistance and plant adaptability [14].

Strawberries have bisexual flowers. The female plants were emasculated and covered by paper bags.

Pollen was collected from the male plants and brought into the stigma of female plants by hand. Newly bred flowers were covered by paper bags to prevent self natural fertilization by insects. After around 2-3 days, they were uncovered to help the hybrid fruit grow and develop in natural conditions. Hybrid fruits were harvested to get their seeds when half the fruit bodies turned red. The seeds were directly sowed in the trays with light and nutritious soil. The soil was watered gently to keep it moist enough for seed germination. The trays were placed in a warm place with proper sunlight for the seeds to sprout. It took the seeds around 15 - 20 days to germinate and 40 - 45 days after to grow up. Finally, the plants were transplanted into the net house to evaluate and select the promising clones. This is a diagram of the cross-breeding and selection process of promising clones.

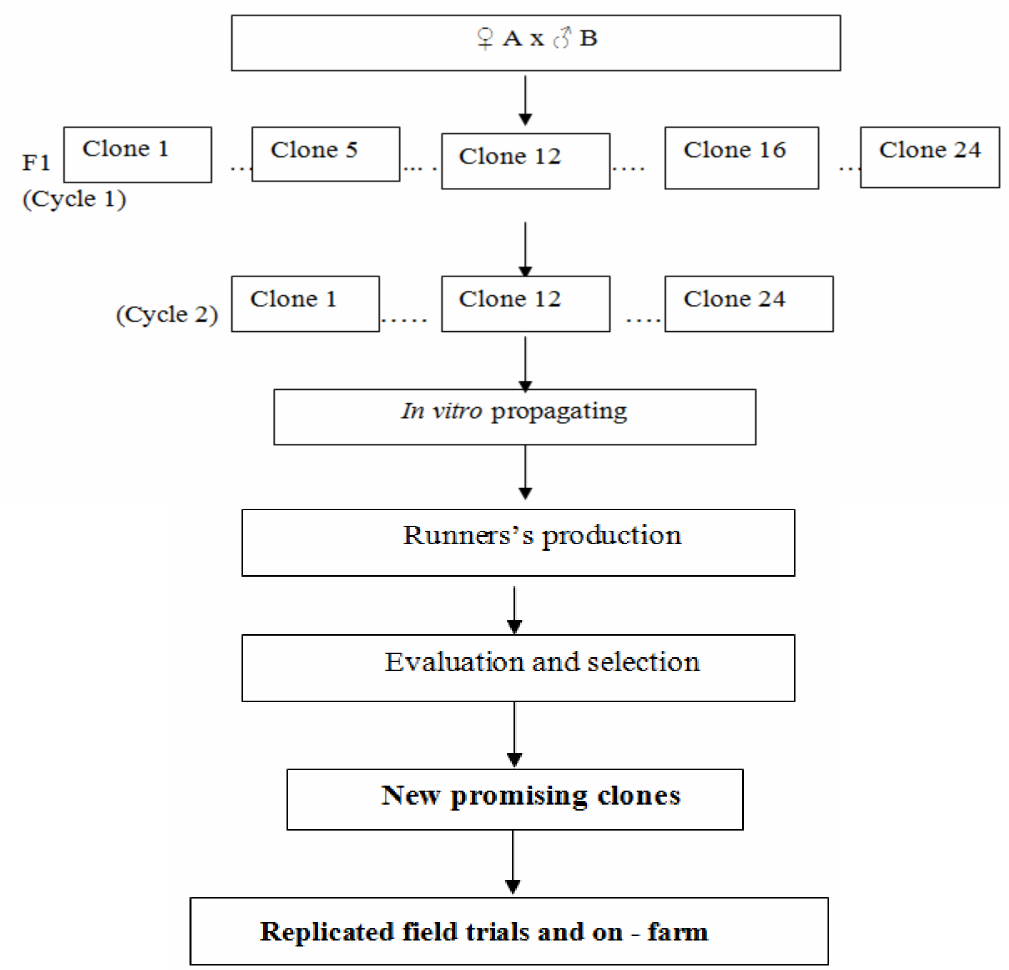

Figure 1. Breeding, selection and evaluation strawberry

2.3.2. Experiment methods

Selection cycles 1, 2 and 3: Experiments were arranged without replication with 50 - 100 plants in 1 plot. The plant's density was 80.000 plants per ha. The control was New Zealand - a common variety produced in the net house conditions in Da Lat city, Lam Dong province.

Experiment design: The experiment was arranged in randomized complete block design with 3 replications and 100 plants in 1 plot. The plant's density was 80.000 plants per ha. The control was New Zealand - a common variety produced in the net house conditions in Da Lat city, Lam Dong province and Bac Ha district, Lao Cai province.

Main selection criteria

*Growth and development indicators

- Plant vigor (1-9): (1) Weak; (5) Intermediate; (9) Vigorous.

- Flowering time (day): Days from transplanting to 50% of plants flowering.

- Ripened fruit time (day): Days from transplanting to fruit ripening.

* Pests and diseases: The assessed methods of pests and diseases in according to National

Technical Regulations QCVN 01-38: 2010/BNN and PTNT [15]:

- Powdery mildew (*Sphaerothe camacularis* f.sp. *fragariae*), Anthracnose (*Colletotrichum fragariae*), and Angular leaf spot (*Xanthomonas fragariae*): (1): Low < 1% of the area of stems, leaves, flowers, and fruits infested; (3): > 1 to 5% of the area of stems, leaves, flowers, and fruits infested; (5): > 5 to 25% of the area of stems, leaves, flowers, and fruits infested; (7): > 25 to 5% of the area of stems, leaves, flowers, and fruits infested; (9) > 50% of the area of stems, leaves, flowers, and fruits infested;

- Thrips (*Frankliniella* spp.), Spider mites (*Tetranichus* spp.): (1) Low (sparse appearance); (2) Medium (distribution of 1/3 leaves, flowers, and fruits; (3) High (distribution > 1/3 leaves, flowers, and fruits);

* Yield components and yield:

- Average number of fruits/plant (fruit): Number of harvested fruits per plot/number of harvested plants per plot.

- Average fruit weight/plant (gram): Weight of harvested fruits per plot/total number of harvested plants per plot.

- Average yield (ton/ha): Collect all fruits in the experimental plot, weigh the weight and convert to ton/ha.

- Average first classified fruit weight (gram): Weight of harvested first classified fruits per plot/Total number of harvested fruits.

- First classified fruit (%): Fruits achieved >10 gram.

* Biochemical qualities:

- Shapes of fruit: Rhombus, Egg, Cone, Heart, Cylinder.

- Firmness levels of fruit: (1): Soft; (2): Medium; (3): Firm.

- TSS (^{0}Brix) (%): measured with the refractometer.

- Taste: (1): Unacceptable; (3): Delicious; (5): Very delicious.

- Flavor: (1): No flavor; (3): Light flavor; (5): Very flavor.

Data analyzing method

The criteria average of each replication of the experiments were calculated by Excel program. Then the SAS 9.1 software was used to compare the data with the Duncan's test at the confidence level of $P < 0.05$.

High-tech technical applications

The experiments were conducted in the 6.0 m height net house conditions which was covered by plastic on the roof and surrounded by protected insect net (32 holes per cm^2);

Plants were grown above the ground on 1.0 m beds (35 cm in width x 30 cm in depth) and mulched by reflective aluminum plastic.

Substrates used for production were the mixture of raw coconut fiber and fresh rice husk according to the ratio of 70:30 (ratio followed by volume). Raw coconut fiber was treated to release tannin and lignin by soaking and rinsing with clean water.

Drip irrigation and automatic fertilizing systems were applied during the growing season.

Strawberry nutrient management

No.	Ingredient	Contain (kg/1000L)	
		Nutrients for the growth season	*Nutrients for the everbearing season*
1	MKP	0.03	0.07
2	KNO_3	0.2	0.2
3	Ca	0.4	0.5
4	$MgSO_3$	0.1	0.1
6	Gold max	0.005	0.005
7	MAP	0.04	0.03
8	Iron Chelate (EDTA) (6%)	0.007	0.007
9	H_3BO_3(17.5%)	0.001	0.001
		EC: 0.6 - 0.8 dS/m; pH: 6.0 - 6.3	EC: 1.1 - 1.4 dS/m; pH 6.0 - 6.3

Adequate mineral nutrition, weather conditions and cultivars decide the yield level of quality production [15]. The plants must be monitored and determined when they need additional nutrient irrigation solutions. Irrigation should be administered at frequent enough intervals to keep the moisture supply even.

+ Growing season (two months before flowering): Nutrient irrigation solution was given 350-400 ml/plant/day, EC: 0.6-0.8. The concentration was gradually increased to 0.1 after each week and not over 0.3 per time to stress the plant.

+ Flowering to everbearing season (from 60 days to 120 days after planting): Nutrient irrigation solution was given 300 - 350 ml/plant/day. EC: 1.1 - 1.4.EC exceeded over 1.5 (EC at the bottom>2.0) leading to sweet fruits but salty plants and dried leaf tips and calyx.

+ Plants were irrigated 6 times per day for 2 - 3 minutes each. The proper time to irrigate depended on weather conditions to adjust. In the everbearing season (everbearing season and fruit harvesting: after 2 months onwards): Watering solution ensured 400 - 500 ml/plant/day and the rate of solution escaping was about 25%; EC: 2.0-2.5.

Planting care

The temperature for strawberries to grow was around 17 - 24°C. The humidity requirement for each stage: 60 - 75% during the growing season, 60 - 65% during the flowering season and 60% for the everbearing season. These were the suitable conditions for strawberries to absorb good nutrients, gain high yield and reduce diseases.

Extra stems and leaves needed pruning regularly to create good ventilation to reduce pests and diseases. Maintaining two stems per one plant and 3 - 4 leaves per one stem to reduce nitrogen in the plants and make the neck's roots create large flower clusters. It helped to stimulate flower sprout differentiation in the everbearing season. Plants remained 5-6 leaves per one stem at the period of flower and fruit.

If there were too many flowers per bundle, small flowers needed to be pruned to achieve large and uniform fruits only. There should have been only 7 - 10 fruits per plant. In addition, bees were put in the flowering stage to improve pollination.

3. RESULTS AND DISCUSSION

3.1. The results of promising clones' selection in cycle 1 (C1)

50 intervarietal crosses with 10.800 seeds and 75 - 80% germination rate was focused on high yield, good quality and resistance to some major diseases. After 45 sowing days, 8,200 seedlings were planted to evaluate and select promising clones in generation 1. Out of the 8,200 seedlings planted, the results selected 18 promising clones with the main characteristics such as vigor growth graded from 8 - 9/9 points, light infection with pests and diseases graded from 1 - 3/9 points as powdery mildew (*Sphaerotheca macularis*), anthracnose (*Colletotrichum fragariae*), angular leaf spot (*Xanthomonas fragariae*), thrips (*Frankliniella* spp.), and spider mite (*Tetranichus* spp.) It took 75 - 80 days after planting (DAP) for the promising clones to flower after the planting and 95 - 100 DAP for the fruits to ripen. Most of the promising clones had fruit bearing types in clustered type except for eight clones that were single. The shapes of fruit were in different forms such as heart, rhombus, egg, cone, cylinder. All fruit turned red when ripened with few seeds, compact, and good firmness. The TSS of all clones was quite high (10.5 - 12.3^{0}Brix). There were 8 promising clones that tasted delicious (PS1.05, PS1.07, PS7.01, PS8.07, PS8.01, PS8.10, PS8.14, PS17.04). Most promising clones tasted light to very flavorful.

The promising clones had the average fruits/plant ranging from 27 - 36 fruits/plant in which first classified fruit reached the ratio of 65.8 to 79.2% and the average weight of first classified fruit was from 10.6 to 14.3 g/fruit. The total yield of promising clones obtained 28.4 to 32.7 tons/ha/year in which six clones achieved over 28 tons/ha/year like PS 1.07 (30.4 tons/ha), PS7.01 (28.4 tons/ha), PS8.07 (29.3 tons/ha), PS8.10 (32.7 tons/ha), PS8.14 (30.1 tons/ha), PS17.04 (31.6 tons/ha).

3.2. The results of promising clone selection in cycle 2 (C2)

18 promising clones in cycle 1 were propagated clonally by tissue culture and produced runners for the second observational trial to evaluate and select the promising clones in cycle 2 (grown during May 2019 to the end of May 2020). During the trial, superior genotypes were selected for the second round as the most promising clones for basic evaluation. The observations showed that the most promising clones, in general, had profuse growth and achieved the vigorous growth of 8 - 9/9 points. Flowering time ranged from 70 to 78 DAP and matured from 94 to 102 DAP. During the growth period, all clones were slightly infected by pests and diseases (Table 1).

Table 1. Some growth characteristics and level of infection by pests and diseases of 18 promising clones in cycle 2 (C2) in May, 2019 to the end of May, 2020

Name of promising clones	Vigor plant (1-9)	Flowering time (day)	Ripened fruit time (day)	Powdery mildew (1-9)	Anthrac-nose (1-9)	Angular leaf spot (1-9)	Thrips (1-3)	Spider mite (1-3)
PS1.01	9	70-75	96-100	3	3	1	1	2
PS1.05	9	70-75	95-100	3	2	1	1	1
PS1.06	9	70-75	96-100	3	2	2	1	1
PS1.07	9	70-75	94-98	2	2	1	1	1
PS3.01	8	72-75	98-102	3	3	1	1	2
PS7.01	9	70-72	96-100	2	2	1	1	1
PS7.02	8	70-72	95-98	3	2	1	1	1
PS8.01	9	75-78	98-102	3	2	1	1	1
PS8.02	9	75-78	96-100	3	2	3	1	1
PS8.03	9	75-78	97-102	3	3	3	1	2
PS8.07	9	75-78	98-100	2	2	1	1	1
PS8.09	8	75-78	97-100	3	2	1	1	1
PS8.10	9	75-80	98-102	2	2	1	1	1
PS8.14	9	75-80	98-102	3	2	1	1	1
PS17.01	8	72-75	96-100	3	3	2	1	1
PS17.02	9	72-74	96-100	3	2	1	1	1
PS17.03	8	72-74	95-100	3	2	1	1	1
PS17.04	9	72-74	94-98	2	1	1	1	1
New Zealand	8	72-74	95-98	4	3	2	1	2

Yield is conditional on the combination of a characteristic list including the number of flowers, and the consequences of fruits such as size, hardiness, and resistance to diseases [16]. The results in table 2 showed that the average fruits/plant of promising clones was 28 - 34 fruits/plant. The fruits were quite large and relatively uniform, leading to a high percentage of first classified fruit. The first classified fruit achieved 64.8 - 80.2%, in which most of the promising clones gained above 70%. In terms of average fruit weight/plant, the promising clones obtained 307.9 - 401.0 g/plant. Out of the 18 promising clones, the six (PS1.07, PS7.01, PS8.07, PS8.10, PS8.14, PS17.04) gave 357.1 - 401.0 g/plant. The total yield of 18 promising clones gained 24.4 - 32.3 tons/ha/year. Six promising clones continued achieving the high yield 28.4 - 32.2 tons/ha/year found in PS1.07 (29.0 tons/ha), PS7.01 (28.8 tons/ha), PS8.07 (29.3 tons/ha), PS8.10 (32.2 tons/ha), PS8.14 (28.4 tons/ha), and PS17.04 (30,1 tons/ha).

Table 2.Yield components and yield of 18 promising clones in cycle 2 (C2) in Da Lat city, Lam Dong province in May, 2019 to the end of May, 2020

Name of promising clones	Fruits/plant (fruit)	First classified fruit (%)	First classified fruit weight (g)	Average fruit weight/plant (g/plant)	Total yield (tons/ha/ year)
PS1.01	30	75.4	12.7	325.3	26.0
PS1.05	32	71.5	13.5	339.5	27.2
PS1.06	30	64.8	14.6	326.6	26.1
PS1.07	29	77.5	14.7	362.0	29.0
PS3.01	31	67.5	13.8	307.9	24.6
PS7.01	33	78.2	13.1	358.2	28.8
PS7.02	30	77.9	11.8	307.9	24.4

PS8.01	34	70.5	11.2	335.6	26.8
PS8.02	33	70.9	12.1	328.2	26.3
PS8.03	33	73.3	11.4	318.7	25.5
PS8.07	31	76.0	12.6	365.9	29.3
PS8.09	31	75.1	12.9	333.4	26.7
PS8.10	32	80.2	13.6	401.0	32.2
PS8.14	33	74.8	12.0	357.1	28.4
PS17.01	34	70.5	12.3	346.9	27.8
PS17.02	33	74.8	11.9	334.1	26.7
PS17.03	28	78.2	12.5	351.8	27.3
PS17.04	34	78.3	12.6	380.3	30.1
New Zealand	33	67.7	11.7	312.7	25.0

Fruit size and color development demonstrate the fruit quality, whereas taste, texture, and flavor attract consumer's acceptance [17, 18]. Therefore, creating and selecting good qualities of strawberry were the most important goal of the research and beautiful fruit appearance will intensely hit the customers. The fruits of promising clones varied deeply and beautifully in shapes that created a variety of fruit shapes such as rhombus, egg, cone, and cylinder. The firmness of fruit decides the shelf life, packaging, and transportation of strawberries. The fruit with higher firmness is more easily accepted by transporters and customers. Most of the promising clones had good firmness, but some were only intermediate with taste and flavor. The TSS (^{0}Brix) of promising clones ranged from 10.2 - 12.3%. Flavor plays an important role in consumers' satisfaction and influences further consumption of fruits in general [19]. The observations showed that the most promising clones expressed the flavor from fragrant to very fragrant (Table 3).

Table 3. Some main biochemical qualities of 18 promising clones in cycle 2 (C2) in Da Lat city, Lam Dong province in May, 2019 to the end of May, 2020

Name of promising clones	Shape of fruit	Firmness (1-3)	TSS (^{0}Brix)	Taste (1-5)	Flavor (1-5)
PS 1.01	Rhombus	3	10.2	3	3
PS 1.05	Rhombus	2	10.4	5	3
PS 1.06	Rhombus	3	12.5	3	3
PS 1.07	Rhombus	2	11.2	5	3
PS 3.01	Cone	2	12.0	3	3
PS 7.01	Egg	3	11.4	5	5
PS 7.02	Egg	2	10.5	3	5
PS 8.01	Cone	2	10.4	5	3
PS 8.02	Cone	3	10.3	3	3
PS 8.03	Cone	2	11.8	3	3
PS 8.07	Cone	3	12.1	5	5
PS 8.09	Cone	3	11.6	3	3
PS 8.10	Cone	3	12.3	5	5
PS 8.14	Cone	3	11.8	5	5
PS 17.01	Rhombus	3	11.0	3	3
PS 17.02	Rhombus	3	11.0	5	3
PS 17.03	Rhombus	3	10.7	3	5
PS 17.04	Rhombus	3	11.3	5	3
New Zealand	Cylinder	3	8.6	3	3

After the evaluation in cycle 2, six promising clones (PS1.07, PS7.01, PS8.07, PS8.10, PS8.14, and PS17.04) gaining high yield and good quality were multiplied and re-evaluated in larger trials in Da Lat city, Lam Dong province and three promising clones (PS8.10, PS8.14, and PS17.04) were assessed in various location (Bac Ha district, Lao Cai province).

3.3. The results of the trial of six promising clones in highland conditions

3.3.1. In Dalat city, Lam Dong province

The trial was carried out from May 2020 to the end of May, 2021 to re-evaluate the potential yield quality and resistance to main pests and diseases of the promising clones in cycle 3. The results showed that the promising clones continued growing strongly and achieved the vigorous growth of 9/9 points. Flowering time was ranging from 70 to 76 DAP and maturing from 94 to 100 DAP. Major pests and diseases were assessed during the growing season. The level of infestation was similar or milder to that of the check New Zealand. Powdery mildew was the major threat of strawberry cultivation directly affected yield and fruit quality. Powdery mildew infection was not significant for the promising clones, ranging from 2 to 3/9 points while the control was more seriously infected (5/9 points). Most of the clones had low levels of infestation by anthracnose and angular leaf spot (2 - 3/9 points). Thrips infection level was not significant for the promising clones while spider mites caused damage at 2/3 points for PS7.01 and PS17.04. The check was seriously infected at 3/3 points (Table 4).

Table 4. Some growth characteristics and level of infection by pests and diseases of 6 promising clones in cycle 3 (C3) in Da Lat city, Lam Dong province in May, 2020 to the end of May, 2021

Name of promising clones	Vigor plant (1-9)	Flowering time (day)	Ripened fruit time (day)	Powdery mildew (1-9)	Anthrac-nose (1-9)	Angular leaf spot (1-9)	Thrips (1-3)	Spider mite (1-3)
PS1.07	9	70-72	94-98	2	2	2	1	1
PS7.01	9	70-72	96-98	3	3	1	1	2
PS8.07	9	74-75	96-100	3	2	1	1	1
PS8.10	9	74-76	96-100	2	2	1	1	1
PS8.14	9	74-76	95-98	3	2	1	1	1
PS17.04	9	72-74	94-98	3	2	1	1	2
New Zealand	8	70-72	95-98	5	3	3	1	3

In this trial, the yield remained high and was comparable to results from generation 2 from previous years. The average fruits/plant of promising clones varied from 31.1 to 34.2 fruits/plant, being the lowest in PS1.07. No significant differences in fruits/plant were found between the remaining clones (33.4 to 34.2 fruits/plant) while the control gave 32.2 fruits/plant. The first classified fruit of promising clones accounted for 76.4 to 82.1%. Among the promising clones, the first classified fruit weight gained from 12.2 to 14.8 g, being the largest in PS1.07. The average fruit weight/plant ranged from 347.6 to 407.9 g/plant. The total yield indicated significant differences between promising clones, in which PS1.07, PS8.07, PS8.10, and PS8.14 continued proving to be the superior clones for high yield (30.2 - 32.6 tons/ha/year) (Table 5).

Table 5. Yield components and yield of 6 promising clones in cycle 3 C3) in Da Lat city, Lam Dong province in May, 2020 to the end of May, 2021

Name of promising clones	Fruits/plant (fruit)	First classified fruit (%)	First classified fruit weight (g)	Average fruit weight/plant (g/plant)	Total yield (tons/ha/ year)
PS1.07	31.1c	77.8b	14.8a	384.1ab	30.7ab
PS7.01	33.6a	76.4b	12.2c	347.6c	27.8c

PS8.07	33.9a	80.2a	12.8bc	388.2ab	31.1ab
PS8.10	33.4a	81.8a	13.6b	407.9a	32.6a
PS8.14	33.4a	82.1a	12.9bc	377.3b	30.2b
PS17.04	34.2a	80.1a	12.3c	372.3bc	29.8bc
New Zealand	32.3b	71.0c	11.9c	320.8d	25.7d
$LSD_{0.05}$	0.8	2.5	0.9	26.7	2.1
CV (%)	5.1	4.8	3.9	5.8	6.9

The top priority of creating new strawberry varieties is high yield in quality berry production as red, sweet, ripe and juicy fruits like those freshly picked from a garden are always the consumers' choice [20]. The main qualities of promising clones were unchanged from generation 2. Most of the promising clones had good firmness except for PS1.07 which gave medium firmness. The TSS (^{0}Brix) had not changed significantly compared to the previous season, graded from 11.2 to 12.6%. In addition, the taste and flavor remained the same as the previous trial (Table 6).

Table 6. Some main biochemical qualities of 6 promising clones in cycle 3 (C3) in Da Lat city, Lam Dong province May, 2020 to the end of May, 2021

Name of promising clones	Shape of fruit	Firmness (1-3)	TSS (^{0}Brix)	Taste (1-5)	Flavor (1-5)
PS1.07	Rhombus	2	11.2	5	3
PS7.01	Egg	3	11.6	5	5
PS8.07	Cone	3	12.3	5	5
PS8.10	Cone	3	12.6	5	5
PS8.14	Cone	3	11.7	5	5
PS17.04	Rhombus	3	11.2	5	3
New Zealand	Cylinder	3	8.8	3	3

Figure 1. The fruits of six promising clones

Figure 2. The field trip to introduce promising clones in Da Lat city, Lam Dong province

3.3.2. In Bac Ha district, Lao Cai province

Three promising clones (PS8.10, PS8.14, PS17.04) introduced by the Potato, Vegetable, and Flower Research Center were planted in Bac Ha district, Lao Cai province at the same time as in Da Lat city. Three promising clones had shown to be relatively well adapted in Bac Ha district. The results showed that the promising clones grew well and achieved the vigor growth of 8 - 9/9 points while the control got 7/9 points. Flowering time ranged from 72 to 75 DAP and matured from 96 to 100 DAP. Powdery mildew infection was not significant for the promising clones (3/9 points) while the control was more seriously infected (5/9 points). PS8.10 and PS8.14 were not significantly infested by anthracnose and angular leaf spot (1/9 points) while PS17.04 was infected in 3/9 points. In this area, thrips infection level was not significant for the promising clones while spider mites caused damage at 2/3 points for PS17.04 and the check (Table 7).

Table 7. Some growth characteristics and level of infection by pests and diseases of 3 promising clones in cycle 3 (C3) in Bac Ha district, Lao Cai province in May, 2020 to the end of May, 2021

Name of promising clones	Vigor plant (1-9)	Flowering time (day)	Ripened fruit time (day)	Powdery mildew (1-9)	Anthrac-nose (1-9)	Angular leaf spot (1-9)	Thrips (1-3)	Spider mite (1-3)
PS8.10	9	73-75	98-100	3	1	1	1	1
PS8.14	8	72-75	98-100	3	1	1	1	1
PS17.04	8	72-74	96-98	3	3	3	1	2
New Zealand	7	70-73	94-97	5	4	3	1	2

In Bac Ha district, yield components and yield are lower than in Da Lat city because Da Lat city is the ideal area for strawberry production in Vietnam. The average fruits/plant of promising clones gained from 17.0 to 18.6 fruits/plant, being the lowest in PS8.14. No significant differences in fruits/plant were found between PS8.10 and PS8.14 (18.0 to 18.6 fruits/plant) while the control gave 17.5 fruits/plant. The first classified fruit of promising clones accounted for 73.6 to 77.0%. The first classified fruit weight in Bac Ha district was smaller than in Da Lat city, gained from 10.6 to 11.4 g, being the largest in PS8.10. The average fruit weight/plant ranged from 158.9 to 172.7 g/plant. Under the climatic conditions in Bac Ha district, the total yield dropped to 1/2 of that in Da Lat city but the yield was acceptable in Bac Ha district conditions. The total yield indicated significant differences between promising clones (12.7-13.2 tons/ha/year), in which PS8.10 continued proving to be the superior clone in Bac Ha district (Table 8).

Table 8. Yield and yield components of 3 promising clones in generation 3 (C3) in Bac Ha district, Lao Cai province in May, 2020 to the end of May, 2021

Name of promising clones	Fruits/plant (fruit)	First classified fruit (%)	First classified fruit weight (g)	Average fruit weight/plant (g/plant)	Total yield (tons/ha/ year)
PS8.10	18.0ab	77.0a	11.4a	172.7a	13.2a
PS8.14	17.0c	75.3a	11.0b	158.9b	12.7b

PS17.04	18.6a	73.6a	10.6c	160.9b	12.9b
New Zealand	17.5bc	66.3b	10.7c	140.6c	11.3c
$LSD_{0.05}$	0.9	3.7	0.3	4.6	0.3
CV (%)	4.7	5.8	4.6	5.2	5.1

The main qualities of the three promising clones in Bac Ha district were unchanged in comparison to those in Da Lat city. All promising clones had good firmness and high TSS (^{0}Brix), from 11.0 to 12.4%. The taste and flavor of PS8.10 and PS8.14 reached 4/5 points while PS17.04 got 3/5 points. The check New Zealand gave 3/5points in taste and flavor (Table 9).

Table 9. Some main biochemical qualities of 3 promising clones in generation 3 (C3) in Bac Ha district, Lao Cai province in May, 2020 to the end of May, 2021

Name of Promising clones	Shape of fruit	Firmness (1-3)	TSS (^{0}Brix)	Taste (1-5)	Flavor (1-5)
PS8.10	Cone	3	12.4	5	5
PS8.14	Cone	3	11.6	4	5
PS17.04	Rhombus	3	11.0	3	3
New Zealand	Cylinder	3	8.4	3	3

After the experiment design, PS8.10 demonstrated to be a remarkably promising clone for high yield (32.6 tons/ha/year in Da Lat city and 13.2 tons/ha/year in Bac Ha district), first classified fruit (77.0 - 82.1%), and TSS (^{0}Brix): 12.4 - 12.6%.

4. CONCLUSION

Under the favorable climatic conditions in Da Lat city, breeding for strawberries is practically feasible that holds a promise for domestic breeding of new cultivars: 10.800 seeds of 50 intervarietal crosses were created and evaluated.

Six promising clones (PS1.07, PS7.01, PS8.07, PS8.10, PS8.14, PS17.04) were selected to apply high technology in the net houses in Da Lat city, Lam Dong province: high yield potential (27.8 - 32.6 tons/ha/year, first classified fruit (76.4 - 82.1%), fruit sweetness (TSS (^{0}Brix): 11.2 - 12.6%). Three promising clones (PS8.10, PS8.14, PS17.04) were selected to apply high technology in the net houses in Bac Ha district, Lao Cai province: High yield potential (12.7 - 13.2 tons/ha/year), first classified fruit (73.6 - 77,0%), fruit sweetness (TSS (^{0}Brix): 11.0 - 12.4%). All promising clones gave good taste, very fragrant and hardness fruit. In addition, they were tolerant to some main diseases such as powdery mildew (*Sphaerotheca macularis*), *Colletotrichum fragariae* and *Xanthomonas fragariae*).

PS8.10 demonstrated to be a remarkably promising clone for high yield (32.6 tons/ha/year in Da Lat city and 13.2 tons/ha/year in Bac Ha district), first classified fruit (77.0 - 82.1%), and TSS (^{0}Brix): 12.4 - 12.6%.

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ISOLATION AND CHARACTERIZATION OF CELLULOSE-DEGRADING BACTERIA FROM THE SPENT MUSHROOM COMPOST

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ABSTRACT

The edible and medicinal mushroom industry in Vietnam is undergoing significant development. Mushrooms are not only a source of clean food but also contain pharmaceutical substances for disease prevention and treatment. Statistics have revealed that the production of 1 kg of mushrooms generates 5 - 6 kg of post-harvest substrates. These substrates contain various polysaccharides, including hemicellulose, cellulose, and residual mycelia. If left untreated, they could contribute to environmental pollution. However, with appropriate treatment, these post-harvest substrates can be transformed into bioproducts, enhancing soil quality and providing nutrients for crops. This study aims to isolate and characterize bacterial strains capable of synthesizing cellulase, an enzyme that breaks down cellulose, from post-harvest substrate compost. The mushroom samples were collected from the mushroom farms in Ha Noi city and Thai Binh province and a total of 44 bacteria strains were isolated. By the agar well diffusion method, 27 strains of bacteria exhibiting cellulase activity were obtained. Notably, the strains FG16 and FG18 had the highest cellulase activity (0.253 U/ml and 1.137 U/ml), respectively. Therefore, these two strains were selected for further experiments. The optimal conditions for cellulase synthesis by strains FG16 and FG18 were found to be as follows: 1.0% and 0.5% CMC added to the culture medium, pH: 5.0 and 7.0, temperatures: 40°C and 45°C, with the raw enzyme collected after 24 hours. Both strains FG16 and FG18 are bacilli, gram positive, positive for MR (Methyl Red), VP (Voges Proskauer) reactions and capable of biosynthesizing some extracellular enzymes such as amylase, chitinase and protease. Based on compairing nucleotide 16S rRNA, strain FG18 closed to strain *Bacillus subtilis* NR112629.1, thus it was called *Bacillus subtilis* FG18

Keywords: *Mushroom compost, cellulose-degrading bacteria, cellulase, Bacillus subtilis.*

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1. INTRODUCTION

Vietnam is an agricultural country, every year the quantity of agricultural by-products reaches millions of tonnes. These by-products are good materials for mushroom cultivation and include rice straws, corn leaves, stems, grasses, etc. The total mushroom production of Vietnam is ranked 9th in the region, equal to 0.3% of China's mushroom production and 0.23% of the world's total mushroom production. The materials for mushroom cultivation are very rich in lignocellulose. Cellulose is the most abundant renewable organic molecule in the biosphere with an estimated annual yield of 4.0 x 10^9 tons. Cellulose is a biological polymer made up of thousands of D-glucose units linked together by β-1,4 glycoside bridges [1]. A large amount of lignocellulose waste is generated through agricultural, forestry and testing processes, especially from agricultural industries such as paper production, textiles, mining and wood processing industries.

However, plant lignocellulose biomass is considered to be biodegradable "waste" and it could be converted into valuable products such as biofuels, chemicals and cheap sources of energy for fermentation, animal food production and human nutrients. The conversion of lignocellulose compounds into fermentable sugars through organism-derived cellulase has been considered as a feasible process and provides potential products to

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minimize the use of fuel, thus, reducing environmental pollution [2]. Raw materials used in mushroom production are often by-products from agro-forestry production such as straw, corn stalks, cotton and sawdust. The production of 1 kg of mushroom would release 5 - 6 kg of post - harvest substrate.

This substrate contains many lignocellulose compounds, nutrients produced when mycelium grows, especially mycelia remaining in the substrate. This is a very good nutrition source for microorganisms to use. Without treatment, this amount of waste would be degraded causing environmental pollution, and negatively affecting the lives of people and farms producing mushrooms because the organic matter would degrade causing water pollution, also spreading a foul smell during decomposition causing air pollution. If it is treated properly, the post-harvest substrate would be converted into bioproducts for not only improving soil quality but also supplying proper nutrition to plants. To solve this problem, microbial strains with strong cellulose-degrading ability are required. Therefore, the objective of this study was to isolate and screen strains of microorganisms that possess cellulose degradation activity to create an initial microbial source for future applied research.

2. MATERIALS AND METHODS

2.1. Material

The bacterial strains using in this article was isolated from posy-harvested mushroom substrate.

Post-harvest substrate samples were collected from Fargreen mushroom farm in Hung Dung commune, Hung Ha district, Thai Binh province and Van Giang mushroom center, located in Lien Nghia commune, Van Giang district, Hung Yen province. Samples were kept in sealed plastic bags and brought immediately to the laboratory for isolation.

2.2. Media

Isolation and maintenance medium for cellulolytic bacteria (g/l) (cellulose agar media): 0.5 g of KH_2PO_4, 0.25 of $MgSO_4$, 2 g of cellulose, 15 g of agar and 2 g of gelatin dissolved in 1000 ml of distilled water at pH 6.8 - 7.2.

LB medium (Luria Bertani): 5.0 g of yeast extract, 10.0 g of peptone and 10.0 g of NaCl, dissolved in 1000 ml of distilled water at pH 7.0 - 7.2.

2.3. Tools and equipment

Tools and equipment used in this study belong to the laboratory Microbial Department – Faculty of Biotechnology – Vietnam National University of Agriculture: Autoclave, technical scale, analytical scale, optical microscope, shaking machine, incubator, oven, pipettes, refrigerator, microwave and pH meter.

2.4. Methods

2.4.1. Methods of isolation, purification

The collected samples were processed and isolation of microorganism strains was conducted following the previously described by Pratima *et al.* (2011) [3]. Ten (10 g) of each sample was mixed thoroughly with 90 ml of sterile distilled water, the solution obtained had a dilution concentration of 10^{-1}. 1 ml of solution at 10^{-1} dilution concentration was taken and diluted with 9 ml of sterile distilled water to make the solution at 10^{-2} dilution concentration. The process of dilution was continued until the solution at 10^{-8} dilution concentration was obtained. Bacterial isolation was carried out on selective media. Pipette was used to take 100 µl of diluted solution at different concentrations (10^{-5}, 10^{-6} and 10^{-7}) and spread on the surface of the agar dish. Petri dishes containing inoculated samples were placed in an incubator at 30^0C, after 2 days colonies were collected. Single colonies were streaked on Petri dishes containing agar and kept in an incubator at 30^oC.

2.4.2. Cellulose hydrolysis capacity of isolated bacterial strains

The bacterial strains were checked by agar well diffusion method on agar plates described by Vo Van Phuoc Que and Cao Ngoc Diep (2011) [4]. Experiments were performed on LB medium-added carboxymethyl cellulose (CMC). Isolated bacterial strains were spotted on plates, and incubated for 2 days at 30˚C. The agar plates were then dyed with Lugol solution. CMC hydrolyzing bacteria would create a colorless area around their colony (halo). Formula to calculate hydrolysis ability (D):

D = [(Halo diameter - Colony diameter)/Halo diameter] x 100

Determination of cellulase activity by quantification of reducing sugar with DNS reagent

Cellulase activity was determined accurately based on the amount of reducing sugar formed after reaction by the spectrophotometric method [5]. Cellulase enzyme acts on the CMC molecule and releases reducing sugar molecules, glucose, into the culture medium. Reagent 3,5-Dinitrosalicylic acid (DNS or DNSA) is yellow, when reacting with reducing sugar, DNS is reduced to 3-amino-5-nitrosalicylic acid (reddish brown) which is capable of absorbing strong light at wavelength 540 nm. The color intensity of the reaction mixture is directly proportional to glucose concentration in the medium. Based on the standard curve of pure glucose with the DNS reagent, the sugar concentration of samples could be calculated.

Effect of cultural conditions on the cellulase-producing ability of selected bacterial strains

Effect of incubation time

Selected bacterial strains were cultures in a liquid medium, shaken for 120 rounds/minute, at 30°C. After 24, 48, 72 and 96 hours, culture media were centrifuged and the raw enzyme was collected. The experiment was repeated 3 times.

Effect of induced substrate concentration

Liquid media-supplied induced substrate (CMC) with different concentrations: 0, 0.5, 1, 1.5 and 2% were prepared. Selected bacterial strains were cultures in liquid medium, shaken for 120 rounds/minute, at 30°C for the optimal culture time surveyed from the previous experiments. After that, culture media were centrifuged and the raw enzyme was collected to determine cellulose activity. Since then, optimal substrate concentration was determined. The experiment was repeated 3 times.

Effect of culture temperature

Culture medium with the optimal substrate concentration as examined above was prepared. Selected strains were grown in the liquid medium with appropriate substrate concentration, and shaken for 120 rounds/minute for optimal culture time, at different temperatures: 25, 30, 35, 40 and 45°C. Culture media were centrifuged and raw enzyme was collected to determine cellulase activity. Since then, the optimal temperature was determined. The experiment was repeated 3 times.

Effect of pH

Selected strains were grown in the liquid medium added substrate with optimal concentration, at optimal temperature as investigated, shaken for 120 rounds/minute. NaOH 1N and HCl 1N were used to adjust initial pH of the culture medium to reach different pH concentrations: 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively.

Selected strains were grown in the liquid medium, and shaken for 120 rounds/minute at the optimal temperature of each strain during the optimal culture time as studied above. Culture media were centrifuged and raw enzyme was collected to determine cellulase activity. Since then, optimal pH was determined. The experiment was repeated 3 times.

Effect of carbon source

Various induced substances including grind husk, dextrin, starch, chitin, xylose, lactose, fructose, D-glucose, mannitol, maltose, sorbitol and CMC were used. pH was adjusted to the optimal value after adding substrates. Selected strains were grown in the liquid medium, and shaken for 120 rounds/minute at optimal temperature and time as investigated in the above experiments. Culture media were centrifuged and raw enzyme was collected to determine cellulase activity. Since then, the optimal carbon source was determined. The experiment was repeated 3 times.

Investigating the ability to degrade print paper of bacterial strains

Strains of bacteria with the strongest CMC hydrolysis ability were selected, and cultured in 50 ml of liquid medium containing 0.1 g of finely ground print paper. After 7 days of culture, paper degrading ability was determined based on the loss of dry print paper. Paper degrading ability was calculated following the formula as below [4].

[(Initial weight – Post weight)/Initial weight] x 100

Similar experiments were conducted to evaluate the ability to degrade straw of bacterial strains which were able to degrade print paper the best. 1.0 g of dry straw was soaked in 100 ml of liquid medium. After 10 days of culture, degrading ability was assessed based on the loss of dry straw. Degrading ability was calculated following the formula as below:

[(Initial weight – Post weight)/Initial weight] x 100

All experimental results were analysed by Excel software vesion 2016.

2.4.3. Molecular identification of the strain FG18

The colony of the strain FG18 was sent to the Phu Sa company to identify based on comparing the nucleotide sequence of the 16S rRNA from the strain FG18 with nucleotide sequences of 16S rRNA of other bacterial strains, deposited on GeneBank (https://blast. ncbi. nlm. nih.gov/Blast. cgi). The phylogenetic tree was conducted by using MEGA 7 software.

3. RESULTS AND DISCUSSION

3.1. Isolation of cellulose-degrading bacteria

Post-harvest mushroom substrate samples were spread on isolation medium supplied with carbon source as CMC. After two days, a preliminary assessment of CMC degrading ability was based on the ability to create a bright ring around the colony. From different post-harvest substrate samples, 44 bacterial strains were isolated, of which 26 strains possessed cellulose-degrading ability with hydrolysis ability from 23.08 to 88.24% (Figure 1 and table 1).

From the above results, we selected two strains of bacteria with the strongest hydrolysis ability FG16 (88.24%) and FG18 (71.43%) to continue further research.

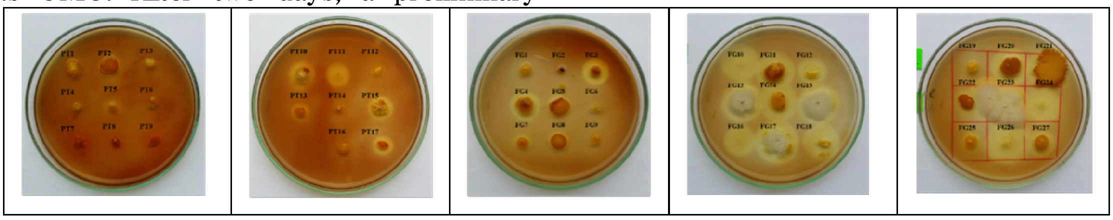

Figure 1. CMC hydrolysis ring created by bacterial strains

Table 1. CMC hydrolysis ability of bacterial strains

Bacterial strain	D	d	Hydrolysis ability (%)	Bacterial strain	D	d	Hydrolysis ability (%)
FG16	1,7	0,2	88.24	FG11	2.2	1.2	45.45
FG18	2.1	0.6	71.43	FG4	1.8	1	44.44
FG3	1.7	0.6	64.71	FG17	2.3	1.3	43.48
FG14	2.2	0.8	63.64	FG21	2.5	1.5	40.00
PT11	1.3	0.5	61.54	FG26	2.7	1.7	37.04
FG10	2	0.8	60.00	FG25	1.1	0.7	36.36
FG24	2.2	0.9	59.09	FG13	2.5	1.6	36.00
FG27	1.8	0.8	55.56	PT15	1.4	0.9	35.71
PT13	1.1	0.5	54.55	FG22	1.2	0.8	33.33
PT17	1.1	0.5	54.55	FG7	1	0.7	30.00
PT10	1.5	0.7	53.33	FG15	2.4	1.8	25.00
FG12	1.7	0.8	52.94	FG5	1.3	1	23.08
FG20	2.4	1.3	45.83	FG8	1.3	1	23.08

D - hydrolysis ring diameter (mm); d - colony diameter

3.2. Effect of culture conditions on cellulase synthesis ability

Effect of incubation time on cellulase activity

Two selected strains were grown in a liquid medium, and shaken for 120 rpm at 30°C in LB broth to investigate the ability to produce cellulase after 24, 48, 72 and 96 hours of culture. OD_{540} value was measured to determine the optimal culture time period for selected bacteria to show the highest activity. The result is shown in figure 2.

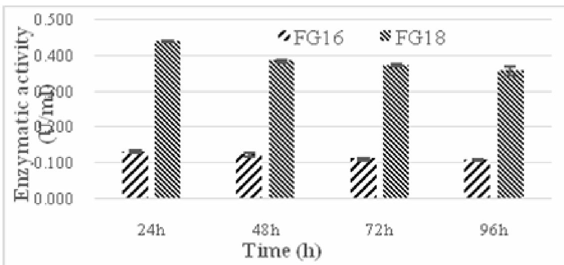

Figure 2. Effect of culture time on cellulase activity

Both studied bacterial strains showed the highest cellulase activity after 24 hours of culture.

Cellulase activity after 48 hours of culture decreased and continued to decrease after 72 and 96 hours. The reason was due to prolonged culture time, nutrient source in the medium was exhausted, and the accumulation of metabolic products would change the initial favorable factors of the environment such as temperature and pH, sometimes accumulating toxins harmful to cells. This would affect the growth of the studied bacterial strain as well as the enzyme production and activity. Farjana Islam and Narayan Roy (2018) when investigating the effect of culture time on the cellulase activity of *Paenibacillus* sp. C1 showed the highest cellulase activity after 24 hours of culture [6]. Our results were similar to that research.

Based on the experimental results, we selected the optimal culture time for the production of cellulase in selected bacterial strains as 24 hours for subsequent experiments.

Effect of concentration of induced substrate on cellulase activity (induced substrate was CMC)

CMC is both a source of carbon nutrition and an inducer for bacteria to produce cellulase. The presence of CMC in the culture medium is useful for cellulase production. Therefore, we studied the effect of CMC concentration in the culture medium on the cellulase activity of selected strains.

Two selected bacterial strains were cultured in LB broth supplemented with CMC substrate at different concentrations: 0, 0.5, 1, 1.5 and 2%, shaken for 120 rounds/minute at 30°C. After 24 hours, the raw enzyme was collected and cellulase activity was determined. The results are shown in figure 3.

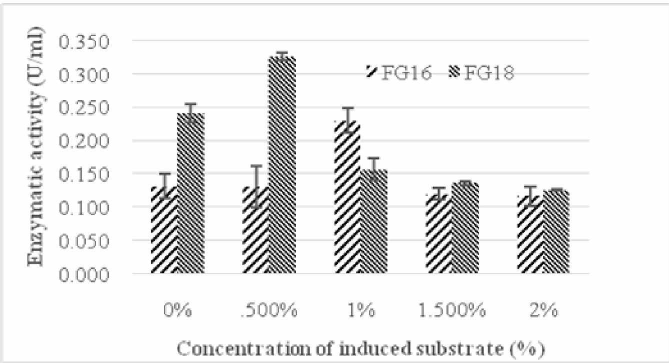

Figure 3. Effect of induced substrate concentration on cellulase activity

The results showed that the highest cellulase activity of strain FG16 was at 1.0% of CMC and strain FG18 was at 0.5% of CMC. When the concentration of induced CMC was increased (1.5%, 2%), cellulase activity decreased. Thus, if the amount of induced substrate (namely CMC) in the culture medium was too high, the growth phase would last longer, the final and secondary products would accumulate more, thus inhibiting the synthesis of cellulose. Our experimental result about strain FG16 were quite similar to the study of Farjana Islam and Narayan Roy (2018) when they investigated the effect of induced substrate concentration (CMC) on the cellulase activity of strain *Paenibacillus* sp. C1, the cellulase activity of this strain was highest at 1.0% of CMC substrate [6]. The research about strain FG18 was similar to the study of Faiz Rasul *et al.* (2015) [7] on strain SM-3M when 0.5% of the induced substrate was applied, cellulase activity was the highest. Therefore, we chose 0.5% of CMC substrate as the optimal substrate concentration for strain FG16 and 1% of CMC substrate for strain FG18 to conduct subsequent experiments.

Effect of temperature on cellulase activity

The culture temperature is a characteristic of the organism and it has a profound effect on the yield and duration of the enzyme synthesis phase [8]. In the range of temperature when the enzyme may exist, when the temperature is increased 10°C, the reaction rate increases twice. When the temperature is too high or too low, it affects the growth of bacteria and the process of cellulase biosynthesis. Therefore, we experimented to determine the effect of temperature on cellulase activity.

Two selected bacterial strains were cultured in LB broth containing 1.0% of CMC substrate for strain FG16 and 0.5% of CMC substrate for strain FG18, shaken for 120 rounds/minute at different temperatures 25, 30, 35, 40, 45 and 50°C. After 24 hours, the raw enzyme was collected and cellulase activity was determined. The results are shown in figure 4.

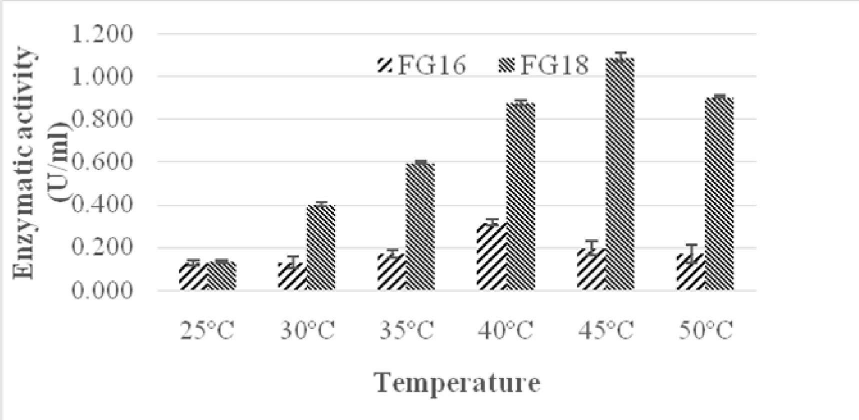

Figure 4. Effect of temperature on cellulase activity

Enzyme's nature is protein, when the temperature is too high or too low, the ability of enzyme biosynthesis and activity of enzyme would be influenced. From the diagram above, the cellulase activity of strain FG16 and FG18 reached the highest values at 40°C and 45°C, respectively.

The result of strain FG16 was similar to the study of Shilpa Lokhande and Pethe AS (2017) [9] about studying the effect of temperature on cellulase activity of bacterial strain *Bacillus thuringiensis*, showing that at 40°C, cellulase activity was the strongest. In the study of Vipul Verma *et al.* (2012), *Bacillus* sp. showed strong cellulase activity when cultured at 45°C. Therefore our result of strain FG18 was quite similar to this study [10].

Effect of pH on cellulase activity

Enzymatic activity strongly depends on pH. pH affects the level of ionization of substrate, the level of ionization of enzymes and the center of enzyme activity, enzyme-substrate complexes and the stability of enzyme proteins [11]. Thus, we chose the pH range from 3 to 11 to do this experiment.

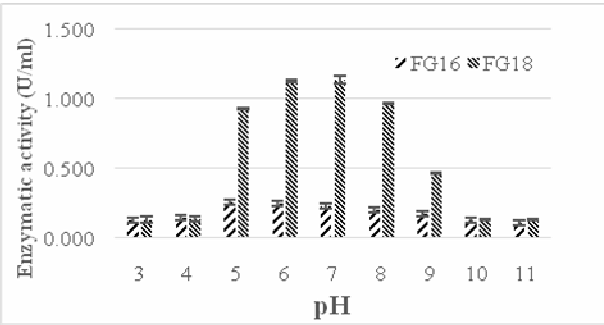

Figure 5. Effect of pH on cellulase activity

Two bacterial strains were cultured in LB broth added 1.0% of CMC as substrate at 40°C for strain FG16, 0.5% of CMC for strain FG18 at 45°C, shaken for 120 rounds/minute at different pH 3, 4, 5, 6, 7, 8, 9, 10 and 11. After 24 hours of culture, raw enzyme was collected and cellulase activity was determined. The results are shown in figure 5.

Cellulase enzymatic activity of two bacterial strains FG16 and FG18 expressed at different pH values, but it was highest at pH 5 and 7, respectively. Therefore, pH of the culture medium greatly affects cellulase biosynthesis ability. Vipul Verma *et al.* (2012), when studying strain *Bacillus* sp., also reported that cellulase activity was the highest at pH 7 [10]. Meanwhile, Bajaj *et al.* (2009) concluded that the cellulase activity of strain M-9 reached the highest value at pH 5 [12]. Our results were similar to previous studies. Therefore, we chose pH 5 and 7 for our two strains FG16 and FG18, respectively, for further experiments.

Effect of carbon source on cellulase activity

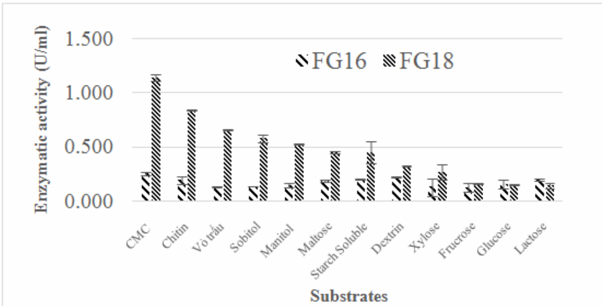

Figure 6. Effect of induction source on cellulase activity

Substrates are substances that have the ability to incorporate into the active center of the enzyme. Induced substrates are the substances that enzymes participating in the reaction catalyze degradation or metabolism of those substrates. These substrates are capable of stimulating the ability to synthesize corresponding induced enzymes. Cellulase is an induced or structural enzyme. In addition, in culture media of enzyme-synthesizing microorganisms, the addition of induced substrates would stimulate microbial enzyme biosynthesis. Therefore, different substrate sources influence enzymatic activity and finding an appropriate source of induced substrate is of great importance.

To find the best-induced substrate for cellulase synthesis, bacterial strain FG16 was cultured at 40°C in LB broth added 1% of different substrate sources and strain FG18 was grown at 45°C in the medium supplied with 0.5% of different substrates. Both strains were shaken for 120 rounds/minute. Different substrates included CMC, grind husk, dextrin, starch, chitin, glucose, fructose, lactose, sorbitol, xylose, maltose and mannitol. After 24 hours of culturing, the raw enzyme was collected and cellulase activity was determined. The results are shown in figure 6.

Various induced substrates of different origins such as CMC, grind husk, dextrin, starch, chitin, glucose, fructose, lactose, sorbitol, xylose, maltose and mannitol showed different effects on the cellulase activity of two bacterial strains. CMC was the best source of induced substrate for the production of cellulase e in both studied strains.

Among different substrates, CMC had the highest ability to stimulate cellulase production [6]. Therefore, our result was similar to previous research. Based on this, we chose CMC as the induced substrate.

Investigating the ability to degrade print paper of bacterial strains

Table 2. Ability to degrade straw and print paper of two bacterial strains FG16 and FG18

Bacterial strain	Print paper degrading ability after 7 days (%)	Straw degrading ability after 10 days (%)
FG16	32.43	18.42
FG18	39.83	19.85

For straw, two strains of bacteria also showed good degrading ability. After 10 days, the degradation was very clear, the ability to degrade straw was 18.42% and 19.85% for FG16 and FG18 (Table 2).

Some results of biochemical tests of FG16 and FG18 are presented in table 3.

Table 3. Results of biochemical tests

Bacterial strain	FG16	FG18
MR reaction	-	-
VP reaction	+	+
Siderophore production	+	+
Chitinase production	+	+
Protease production	+	+
Citrate reaction	+	+

3.3. Molecular identification of the strain FG18

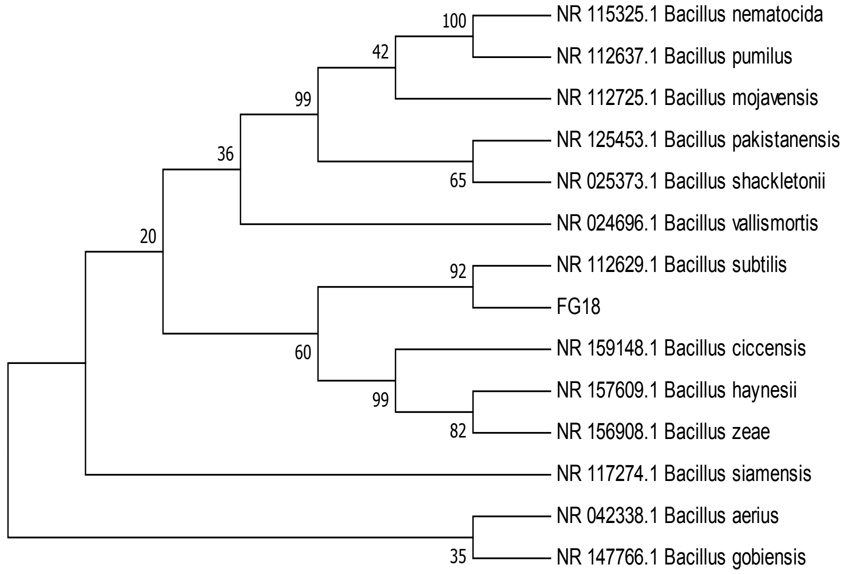

Figure 7. Phylogenetic tree of strain FG18

16s rRNA gene of strain FG18 was sequenced at Phu Sa company and compared with the 16s rRNA sequence of bacteria on the gene bank by BLAST software. The result showed that the 16s rRNA sequence of strain FG18 was 99% similar to strain *Bacillus subtilis*. Therefore this strain was named as *Bacillus subtilis* FG18.

4. CONCLUSION

After growing mushrooms on substrate samples, we isolated 26 strains that possessed cellulose - degrading ability with hydrolysis ability ranging from 23.08 to 88.24% and selected strain FG16 and FG18 with the highest cellulase activity, 0.253 U/ml and 1.137 U/ml, respectively. These two strains were able to degrade straw and paper.

Strain FG16 synthesized cellulase enzyme most strongly when it was cultured in LB broth containing 1.0% of CMC substrate, at pH 5, 40°C, shaken for 120 rounds/min for 24 hours. FG16 was capable of degrading print paper and straw.

Strain FG18 was able to degrade straw and paper. FG18 synthesized cellulase enzyme most strongly when cultured in LB broth containing 0.5% of chitin as substrate, at pH 7 and 45°C for 24 hours. This strain was 99% homologous to the strain *Bacillus subtilis* NR112629.1, thus it was called *Bacillus subtilis* FG18.

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STUDY ON THE EFFECT OF ENZYME TREATMENT CONDITIONS ON EXTRACTION EFFICIENCY AND MACADAMIA *(Macadamia integrifolia)* OIL QUALITY

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ABSTRACT

Macadamia nut oil is a source of healthy edible oils with a high content of unsaturated fatty acids and rich in bioactive compounds. Recent research has focused on developing new methods for extracting oil from macadamia nuts to enhance quality and improve oil extraction efficiency. In this study, we evaluated the enzyme treatment conditions to obtain higher oil yield and better oil quality from macadamia nuts. Specifically, we analyzed oils yield (%), peroxide value, acid and iodine value, saponification value, fatty acid composition, and sensory evaluation. The results showed that,among three enzymes tested (Viscozyme® L, Pectinex® Ultra SPL, Celluclast® 1.5L), Viscozyme® L was selected due to its superior oil extraction efficiency. Viscozyme® L enzyme demonstrated higheffectiveness when the particle fragment size was< 2 mm, combined with a treatment concentration of 0.5%, a treatment duration of 2 hours, and the addition of water at a rate of 30% (v/w – volume compared to weight of macadamia nut). Under these optimized conditions, the oil yield reached approximately 65 to 66%, a significant improvement compared to other treatment conditions yielding only about 15 to 20%. Furthermore, the acid and peroxide values were lower, and the oil exhibited favorable sensory characteristics. Notably, the fatty acid composition of the oil remainedunaffected by the enzymatic treatment. Consequently, this enzyme treatment method can be employed before pressing to increase the oil extraction yield and enhance the oil's quality, making it suitable for development and application in the functional food, cosmetic, and pharmaceutical industries.

Keywords: *Fatty acids, green technology, macadamia oil, functional foods, enzyme processing.*

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1. INTRODUCTION

In recent years, the cultivation of macadamia nuts has become popular in the Northwest and Central Highlands regions of Vietnam. Vietnamese macadamia nuts are now being exported to many countries worldwide, primarily as dried macadamia nuts and seeds. However, valued-added products like macadamia nutritional drinks, oil, cake, candies, and chocolate are imported mainly from abroad. Some companies have experimented with macadamia nut oil production in the Central Highlands, particularly in Dak Nong. Nevertheless, most production facilities currently utilize screw presses and employ high-temperature pressing methods. Unfortunately, this approach can lead to the decomposition of the nutritional quality and sensory value and potentially yielding substances harmful to consumers' health. Macadamia oil is a crucial product derived from macadamia nuts, known for itshigh content of monounsaturated fatty acids (approximately 80%) and the presence of bioactive compounds such as tocopherols, squalene, and phytosterol [1]. It is essential to note that the efficiency of oil recovery, the nutritional value, and the physicochemical properties of the oil are greatly influenced by the chosen oil extraction method [2].

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Currently, several methods for extracting oil from macadamia nuts are employed, including screw pressing, the use of solvents like hexane, ethanol, oil ether, and butanol, low-temperature hydraulic pressing, supercritical carbon dioxide, ultrasound, microwave, and enzymatic hydrolysis methods. Each of these methods has its own set of advantages and drawbacks. The solvent-based extraction method yields a high quantity of oil but often leads to environmental pollution and can adversely affect the quality of the oil. Consequently, various alternative extraction techniques have been researched to provide safe and environmentally friendly alternatives. One such method is supercritical CO_2, a modern approach known for its high oil extraction efficiency and eco-friendliness. However, this method can be costly, and the high pressure involved may reduce the presence of bioactive ingredients in the oil [3]. The traditional pressing method is characterized by its low cost and ease of implementation, resulting in oil with a bright color, characteristic aroma, and stable quality [4, 5]. However, this method can harm the bioactive components of macadamia oil when conducted at high temperatures. Therefore, the cold-pressing method is often considered one of the most advantageous and recommended. Nevertheless, oil extraction through the low-temperature pressing method yields low oil recovery efficiency, typically around 30 - 40%, approximately 25 - 50% lower than the solvent-based method [6].

Exploring pretreatment methods before pressing is essential to enhance oil recovery efficiency in cold pressing. Some critical forms of treatment include crushing, reducing kernel size, and using heat or non-heat treatment to break or thin the cell wall, thereby facilitating the pressing process.

Employing the microwave method for treating raw materials before oil extraction is an effective and economically viable solution. Microwave treatment generates a high-frequency current that breaks the cell wallsthrough an impulse reaction. The rapid heating and vibration lead to cell rupture, effectively supporting the oil extraction. Before cold pressing (hydraulic), microwave pretreatment can boost oil extraction efficiency by up to 50% [5]. However, it's worth noting thatthis method may not be suitable for raw materials rich in biological compounds.

As the trend of consuming functional foods that support human health has increased in recent decades, it has become crucial to ensure that the extraction of macadamia nuts maintainsthe biologically active compounds and nutrients they contain. Macadamia nuts have ahigh content of unsaturated fatty acids, which benefit one's health. Using enzymes to support the extraction process is a suitable and environmentally friendly solution for oil extraction. Enzymatic pretreatments to enhance oil recovery and maintain quality in the extraction process have been the subject of prior investigations. De Aquino*et al.* (2019) reported that the enzyme method for extracting sunflower seed oil yielded similar results to the solvent-based extraction method [7]. They tested the Alcalase endo-protease enzyme to optimize oil extraction conditions from pine kernels [8]. In addition, Balvardi*et al.* (2015) suggested an environmentally friendly method using two enzymes, Alcalase 2.4L and Celluclast 1.5, to extract oil from wild almonds [9]. The primary purpose of using enzymes is to hydrolyze and aid in the breakdown of plant cell walls, thereby increasing the efficiency of oil extraction while preserving the quality and bioactive ingredients in macadamia nuts.

Nevertheless, there currently needs to be more references in the country regarding the applications of enzyme processing for extracting oil from macadamia nuts. In this study, we evaluate the enzyme treatment for oil extraction efficiency and the quality characteristics of macadamia nut oil andpropose an effective application process for macadamia nut oil extraction. We have comprehensively analyzed and compared the resulting macadamia oil's physicochemical properties, fatty acid composition, peroxide value, acid value, and organoleptic properties.

2. MATERIALS AND METHODS

2.1. Materials

Macadamia materials: Macadamia fruits (*Macadamia integrifolia*) were harvested from various farms in Dak Nong province in 2022 and 2023.

Preparation of macadamia nut samples: After harvesting, the fruits are gathered, and their outermost green skin is removed. Once peeled, the macadamia nutsundergo a drying process: initial drying for two days at room temperature (26 -28°C).

Subsequent drying at 35°C for three days. Finally, they are placed at 50°C in a drying oven (SANYO DRYING OVEN, MOV-112, Capacity: 97 L, Sanyo Electric Co., Ltd., Japan) and dried until the desired dryness level is achieved. After this final stage, the husks are removed, leaving only the kernels, which are then used for research experiments.

In the current experiment, three enzymes were used, as follows:

Enzyme type	Commercial name	Source	Technical properties	Composition
Enzyme 1	Viscozyme® L	Cell Wall Degrading Enzyme Complex from *Aspergillus* sp. Lysing Enzyme from *Aspergillus* sp.	Storage temperature: 2-8°C; Appearance: transparent, liquid; Color: light brown; Products of Novozyme Corp; Activity: ≥100 FBGU/g; Density: ~1.2 g/mL at 25°C (lit.).	It contains a variety of carbohydrases, including arabanase, cellulase, glucanase, hemicellulase, and xylanase.
Enzyme 2	Pectinex® Ultra SPL	Pectinase from *Aspergillus aculeatus.*	Storage temperature: 2-8°C; Appearance: transparent, liquid; Color: light brown; Products of Novozyme Corp	Pectinase
Enzyme 3	Celluclast® 1.5L	Cellulase from *Trichoderma reesei* Generic name: 1,4-(1,3:1,4)- β-D-Glucan 4-glucano- hydrolase Celluclast® 1.5L.	Storage temperature: 2-8°C; Appearance: transparent, liquid; Color: light brown; Products of Novozyme Corp.	Cellulase

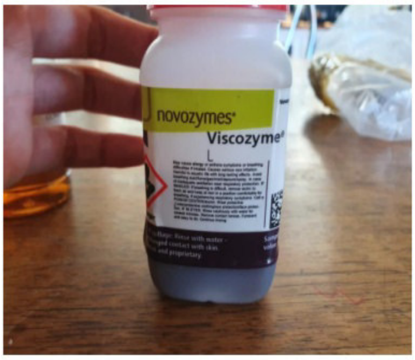

Figure 1. Preparation of materials

The macadamia oil pressing machine used in this study is ahydraulic oil press, which functions as a cold press oil machine. It has a batch capacity of 5 kg, equivalent to 20 kg/hour. The device offers a temperature range of 25 to 100°C. Vina Nha Trang Mechanical Joint Stock Company provided this equipment, and additional equipment details can be found in figure 2.

The centrifuge device used is aHermle Z323 model with a 4 x 100 ml capacity. It is equipped with high-speed micro rotorscapable of reaching speeds up to 17.000 rpm or 26.810 xg. The device offers a temperature range from -20 to 40°C. This centrifuge is manufactured in Germany; additionalequipment details are shown in figure 2.

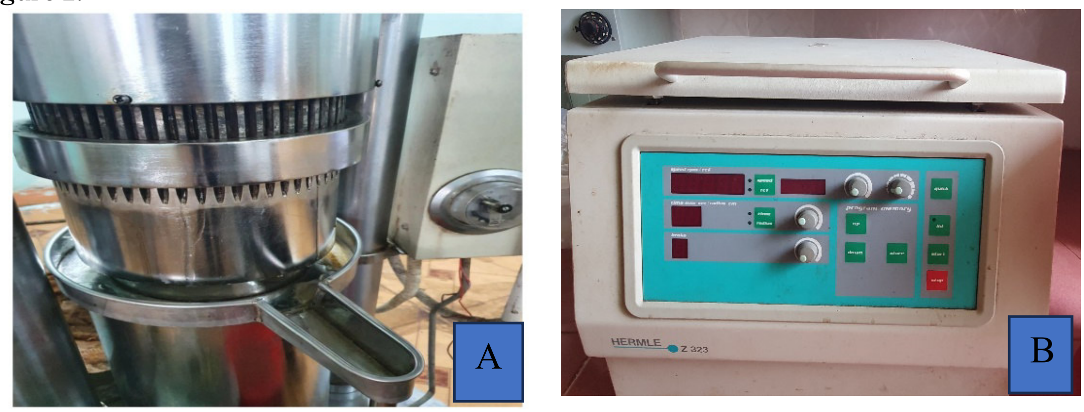

Figure 2. Hydraulic oil press (picture A) and centrifuge device (Picture B)

2.2. Research methods

2.2.1. Experimental design

To conduct experiments on the effect of enzyme treatment on extraction efficiency and the quality of macadamia oil and to determine optimal enzyme-assisted conditions on the oil extraction process, the experiments are set up according to figure 3 as follows:

Experiment 1: Effect of particle size and enzyme type on extraction efficiency and quality of macadamia oil

3 particle sizes: A1- 4 - 6 mm; A2- 2-4 mm; A3- <2 mm.

3 enzymes: Viscozyme® L, Pectinex® Ultra SPL, Celluclast® 1.5L.

In this experiment, the macadamia nutsare treated with enzymes at a fixed concentration of 0.5%. The pretreatment time is set at 1 hour, and the pressing is conducted at approximately 25°C. Each treatment involves 1 kg of milled macadamia nut.

Experiment 2: Effect of enzyme concentration

In the experiment, four different enzyme concentration levels of 0.3%, 0.4%, 0.5%, and 0.6% are compared with a control without enzymes. All treatments use macadamia nut powder with less than 2 mm particle size. Each treatment involves processing 1 kg of milled macadamia nuts. This setup aims to analyze the impact of varying enzyme concentrations on the macadamia oil extraction process when working with fine nut powder.

Experiment 3: Evaluating the impact of enzyme treatment time and water ratio

In this experiment, macadamia nut material, milled to a size of <2 mm, is subjected to soaking and treatment with 0.5% Viscozyme® L enzyme. This treatment varies in reaction time, set at three intervals of 1 hour, 2 hours, or 3 hours. Additionally, water is added at three different levels of 20%, 30%, and 40%, depending on the treatment. Each treatment involves processing 1 kg of milled macadamia nut. The aim is to assess the effects of enzyme treatment time and water ratio on the macadamia nut material.

Experiment 4: Investigating the impact of pressing temperature

In this experiment, the pressing temperature is varied within the ranges of 25 - 40°C (precisely 25, 30, 35, and 40°C). Macadamia nut material, milled to a size smaller than 2 mm, is soaked and treated with 0.5% Viscozyme® L enzyme. Additionally, water is added at a consistent level of 30%. The reaction can proceedfor 2 hours, and each treatment involves 1 kg of milled macadamia nuts. The objective is to assess the influence of different pressing temperatures on the macadamia nut material.

2.2.2. Analyzing methods

The formula for calculatingoil recovery efficiency (Oil Yield%) is as follows:

Oil yield (OY, %) = (Material weight before pressing - Material weight after pressing) *100/Percent oil in the material.

Peroxide value: TCVN 6121: 2018 (ISO 3960: 2017) [10].

Acid value: TCVN 6127: 2010 (ISO 660: 2009) [11].

Saponification value: TCVN 6126: 2015 (ISO 3657: 2013) [12].

Iodine value: TCVN 6122: 2015 (ISO 3961: 2013) [13].

Oil content: AOAC 2003.05 and AOAC 2003.06 [14].

Fatty acid composition in macadamia oil: TCVN 9675: 1 (ISO12966-1) and TCVN 9675: 2 (ISO12966-2) [15].

Sensory evaluation: Employing the descriptive method, establish an assessment committee comprising five individuals with expertise in sensory evaluation. Develop sensory criteria using an illustrative table, conduct pre-assessment training for evaluation levels, outline assessment methods, and implement a scoring system. Calculate the average score from committee members using a scale of 1 to 4 (with 1 indicating the lowest and 4 signifying the highest preference or quality).

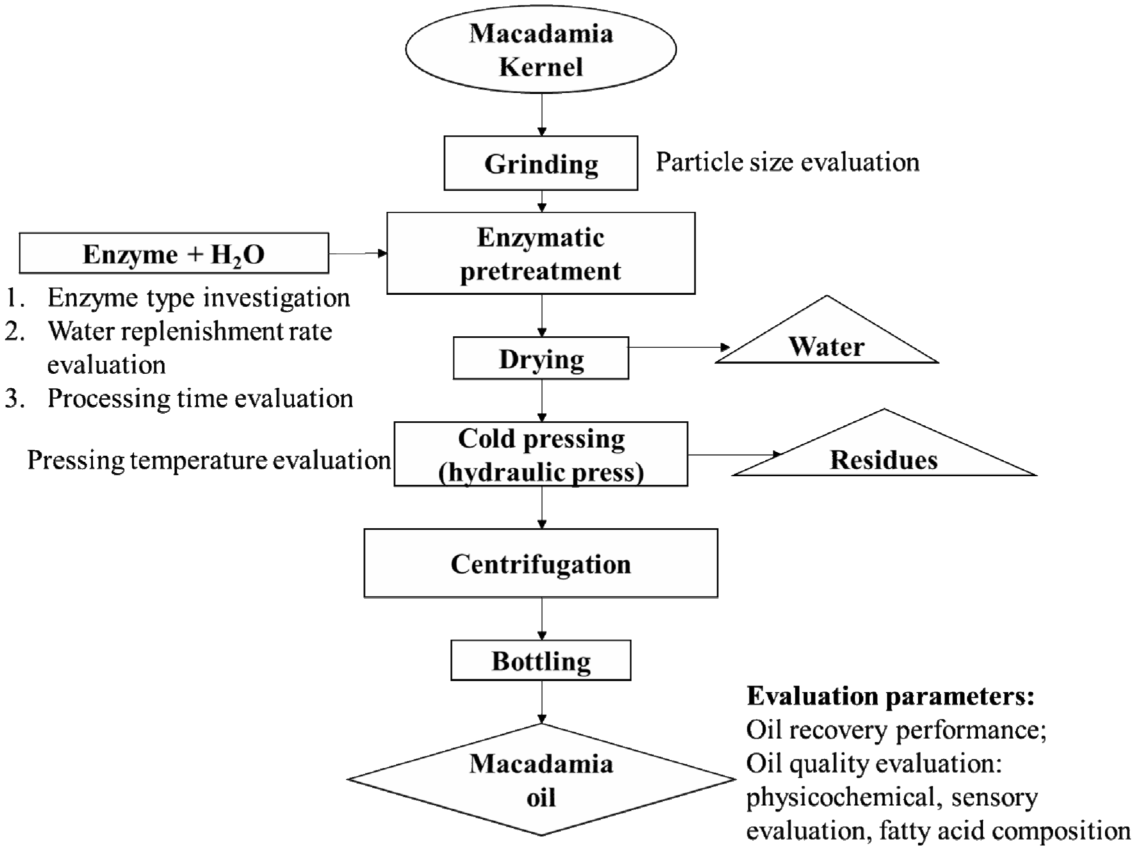

Figure 3. Diagram of the proposed macadamia oil extraction process

Statistical analysis

The experimental results were conducted with three replicates and subjected to statistical processing using IBM SPSS statistics 26.0 software for Windows (SPSS Inc., Chicago, USA). Significant differences between treatments were assessed through one-way analysis of variance (One-way ANOVA), with the Tukey test considering significance at $p < 0.05$. The data are expressed as the mean (Mean) of three replicates ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Effect of particle size and enzyme type on macadamia oil extraction efficiency and quality

Table 1 displays the results of evaluating the extraction efficiency of macadamia oil using different commercial enzymes and 03 distinct particle sizes through the hydraulic pressing method.

Table 1. Oil extraction efficiency by formulas (OY, %)

Factor A (particle size)	Factor B (type of enzyme)			
	B1 (Viscozyme® L)	B2 (Pectinex ultra spl)	B3 (Cellulast)	Control (CTL)
A1 (4 - 6 mm)	48.72 ^{Ca} ± 0.51	42.01 ^{Cb} ± 0.76	43.88 ^{Cb} ± 1.01	34.66 ^{Cc} ± 0.28
A2 (2 - 4 mm)	52.72 ^{Ba} ± 0.27	46.53 ^{Bc} ± 0.35	47.48 ^{Bb} ± 0.21	37.07 ^{Bd} ± 0.25
A3 (< 2 mm)	59.11 ^{Aa} ± 0.79	51.58 ^{Ab} ± 0.54	51.67 ^{Ab} ± 0.58	39.74 ^{Ac} ± 0.25
Medium	53.52 ^{a} ± 4.56	46.71 ^{b} ± 4.18	47.68 ^{b} ± 3.43	37.16 ^{c} ±2.21

A, B, C, D: Represent values with statistically significant differences ($p < 0.05$) in the same column; a, b, c, d: Represent values that have a statistically significant difference ($p < 0.05$) in the same row. *(Note: The difference in the Levene test is greater than 0.05, so the variance of the group of values is the same. The difference of F-test in both enzyme type, particle size, and enzyme*size is less than 0.05, thus showing a difference in oil extraction efficiency in different enzymes and particle sizes as well as between different enzymes and particle sizes).*

Table 1 illustrates the significant impact of enzyme type and particle size on the extraction efficiency of macadamia oil. The results showed that amongst tested enzymes, Viscozyme® L outperformed the others, yielding an average extraction efficiency of 53.52%, which was significantly higher (*p<0.05*) compared to Pectinex ultra spl (46.71%) and Cellulast (47.68%). In contrast, the control sample without enzymes yielded only 37.16% oil extraction efficiency.

Furthermore, the results in table 1 highlight a substantial difference in oil extraction efficiency based on particle size, with a cleartrend of increased efficiency as the particle size decreases. Specifically, particle sizes smaller than 2 mm consistently performed better than larger flakes in all formulations. When the particle size was < 2 mm, the highest oil extraction efficiency was achieved, with 59.11% when treated with Viscozyme® L, 51.58% with Pectinex ultra spl, 51.67% with Cellulast, and 39.74% with CTL (without enzymes). It is evident that all three types effectively contribute to the breakdown of the cell wall of oil-containing granules. Viscozyme® L enzyme exhibited the highest initial efficiency, while there was no significant difference between Cellulast and Pectinex Ultra spl enzymes.

Initial evaluation showed that pretreating the seeds with Viscozyme® L enzyme and using macadamia particles smaller than 2 mm resulted in optimal support for hydraulic macadamia oil pressing, leading to a substantial improvement in oil extraction efficiency, approximately 20% higher compared to non-enzymatic treatments.

The higher oil extraction efficiency of Viscozyme® L enzyme can be explained as it contains a variety of carbohydrases such as arabanase, cellulase, glucanase, hemicellulase, and xylanase. These enzymes work synergistically to facilitate the release of oil constituents from the cell by breaking down hemicellulose, cellulose, and pectin molecules, ultimately breaking down cell wall components.

The use of enzymes to enhance oil extraction and the preservation of active ingredients has been extensively researched, proposed, and applied. However, it's essential to recognize that each type of oil-containing seed possesses a unique composition and structure within the oil-containing cell wall. Therefore, to optimize the extraction process for each specific type of oil-containing seed, the selection of enzymes and suitable conditions must be tailored accordingly. Typically, the components surrounding the oil include pectin, cellulose, hemicellulose, protein, and more. This is why mixed enzymes like Viscozyme® L proved more effective in supporting oil separation from seeds. The results of our experiment align with previous studies that have compared the impact of enzyme treatment on oil recovery from sunflower seeds. Specifically, Latif and Anwar (2009) conducted a similar comparison, evaluating the efficiency of 05 different enzymes (Protex 7L, Alcalase 2.4L, Viscozyme® L, Natuzyme, and Kemzyme). Viscozyme® L exhibited the highest oil extraction efficiency at 39.7 ± 0.4%, significantly greater than the non-enzymatic treatment (18.3 ± 0.3%) [16].

Table 2. Physicochemical properties of macadamia oil

Physicochemical properties	Factor A (size)	Factor B (type of enzyme)			Mean	CTL
		B1 Viscozyme® L	B2 Pectinex Ultra spl	B3 Cellulat		
Acid number (mgKOH/g)	A1 (4 - 6 mm)	0.50 ± 0.02	0.53 ± 0.01	0.53 ± 0.01	0.52 ^{C} ± 0.02	0.55 ^{C} ± 0.03
	A2 (2 - 4 mm)	0.49 ± 0.01	0.51 ± 0.02	0.50 ± 0.02	0.50 ^{B} ± 0.01	
	A3 (< 2 mm)	0.47 ± 0.01	0.49 ± 0.01	0.48± 0.01	0.48 ^{A} ± 0.01	
	Mean	0.49 ^{A} ± 0.02	0.51 ^{B} ± 0.02	0.50 ^{B} ± 0.02		
Peroxide value (meq/kg)	A1 (4 - 6 mm)	1.32 ±0.05	1.48 ±0.04	1.46 ±0.04	1.42 ^{B} ±0.08	1.59 ^{C} ±0.01
	A2 (2 - 4 mm)	1.29 ±0.04	1.32 ±0.03	1.30 ±0.03	1.30 ^{A} ± 0.03	
	A3 (< 2 mm)	1.24 ±0.04	1.28 ±0.04	1.27 ±0.04	1.26 ^{A} ±0.04	
	Mean	1.28 ^{A}±0.05	1.36 ^{B}±0.09	1.34 ^{B}±0.09		
Iodine value (gIod/100 g)	A1 (4 - 6 mm)	74.6 ± 0.72	74.8 ± 0.67	74.7 ± 0.53	74.5 ^{A}± 0.57	74.4 ^{A}± 0.07
	A2 (2 - 4 mm)	74.4 ± 0.74	74.7 ± 0.29	74.8 ± 0.84	74.7 ^{A}± 0.60	
	A3 (< 2 mm)	75.1 ±0.51	75.0 ± 0.41	74.8 ±0.22	75.0 ^{A}± 0.37	
	Mean	74.7 ^{A}± 0.65	74.8 ^{A}± 0.44	74.8 ^{A}± 0.55		
Saponification value (mgKOH/g)	A1 (4 - 6 mm)	197 ±0.86	198 ± 0.35	197 ± 0.82	197 ^{A}± 0.76	198 ^{A}± 0.68
	A2 (2 - 4 mm)	196 ±0.93	197 ± 0.76	196 ± 0.74	196 ^{A}± 0.74	
	A3 (< 2 mm)	197 ± 1.06	198 ± 0.69	198 ± 0.94	198 ^{A}± 0.83	
		197 ^{A}± 0.96	198 ^{A}± 0.8	197 ^{A}± 0.96		
Refractive value at 20°C $^{(s/c)}$	A1 (4 - 6 mm)	1.466	1.466	1.466	1.466 ^{A}	1.466 ^{A}
	A2 (2 - 4 mm)	1.466	1.466	1.466	1.466 ^{A}	
	A3 (< 2 mm)	1.466	1.466	1.466	1.466 ^{A}	
		1.466 ^{A}	1.466 ^{A}	1.466 ^{A}		

A, B, C, D: Represent values with statistically significant differences (p<0.05) in the same column or in the same row. (*Note: The difference of F test in enzymes and particle size to acid value and peroxide value is less than 0.05, thus showing a statistically significant difference in acid value and peroxide value in different types. Different enzymes, different particle sizes. However, there was no difference between enzymes on acid value according to particle size).*

Furthermore, several quality parameters were analyzed to evaluate the impact of the enzyme and particle size on the quality of the macadamia nut oil, including the value of acid, peroxide value, iodine value, saponification value, and refractive value. The analytical results in table 2 show that particle size and the type of enzyme used influence the acid value and peroxide value. At the same time, they do not significantly affect the iodine value, saponification value, or refractive value. As particle size decreases, there is a noticeable reduction in the acid value and peroxide value, indicating an improvement in the quality parameters of the oil. This suggests that smaller particle sizes result in better oil quality.

Additionally, among the three enzymes tested, Viscozyme® L positively affected oil quality, with lower acid and peroxide values compared to the other two enzymes and even the control sample. In contrast, there was no significant difference between Cellulast enzymes and Pectinex Ultra spl enzymes. The particle size is one of the main factors influencing both oil pressing efficiency and the quality of macadamia oil. Uniformly ground particle size contributes to higher quality and improved performance during pressing. Smaller particle sizes lead to more significant destruction of oil-containing cells and the easy release of oil in its free form. However, it's worth noting that the crushed powder particles are tiny, they can tend to clump during enzyme treatment, and the resulting powder may not be porous enough during the drying process. This, in turn, can reduce the efficiency of the oil extraction process [17]. Moreover, if the seeds are crushed into tiny particles, the greater surface area exposed to oxygen and the microflora can negatively impact the quality of the oil. This is especially true if no prompt and effective treatment [18].

Conversely, larger particle size reduces contact between the material and the enzyme. Cells located deep within the seedmay not undergo the necessary changes under the influence of the enzyme, leading to less efficient oil extraction from these regions [19]. Considering the color and taste of the oil after pressing, it exhibits a light yellow color, a greasy taste, and the characteristic aroma of macadamia oil. Notably, the organoleptic quality of the oil obtained through treatment did not differ from that of the control. Therefore, based on these considerations, Viscozyme® L enzyme and a particle size smaller than 2 mm were selected for further experiments.

3.2. Effect of enzyme concentration

The experimental results show that the oil extraction efficiency significantly increased at all tested concentrations after 1 hour of enzyme treatment (Figure 4). With a gradual increase in enzyme concentration, the oil extractionefficiency likewise increased progressively. At concentrations of 0.5% and 0.6%, the highest oil recovery was achieved at 59.4 and 60.7%, respectively, with no statistical difference between these two concentrations (*$p>0.05$*). In contrast, the control formula without enzyme treatment resulted in an oil recovery efficiency of only 39.5%. The non-enzymatic procedure with added water achieved an oil extraction efficiency of 42.1%, with no significant difference compared to the control sample (Figure 4).

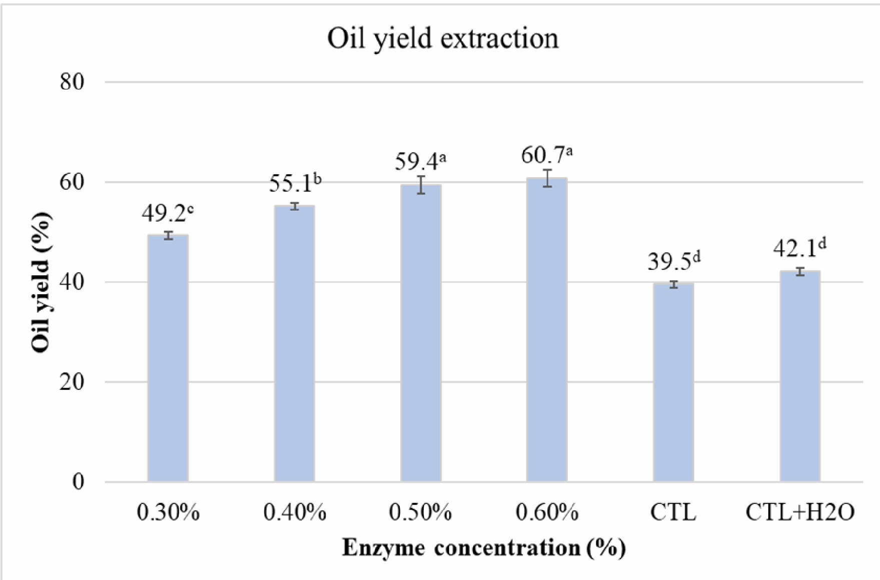

Figure 4. Oil extractionefficiency at different concentrations of Viscozyme® L enzyme (%)

a, b, c, d: Indicate values that have a statistically significant difference ($p<0.05$).

Several studies have mentioned the effect of enzyme addition on oil extraction efficiency and have similar results. For instance, Ezeh *et al.* (2016) concluded that, when comparing different seed pretreatment methods before pressing, the enzyme method resulted in the highest oil recovery yield, recovering approximately 90% of the oil contained in the oil in the seeds, while high-pressure treatment showed almost no increase in yield compared to the control [20]. Other studies have also confirmed that enzyme treatment enhances oil extraction efficiency from peanut seeds [21, 22]. Notably, among the enzymes used, Alcalase demonstrated the highest oil extraction efficiency, reaching around 73.45%, compared to the control treatment without enzymes, which yielded only 30.59%.

Furthermore, the acid and peroxide values were analyzed to provide an initial assessment of the quality of the obtained oil based on the preceding experimental results. These two parameters are crucial indicators of the oil quality and are susceptible to change depending on processing conditions, whereas the other values remain relatively stable.

In particular, the acid and peroxide values slightly decreased with increasing enzyme treatment concentration from 0.3-0.6%, although the difference was not statistically significant. However, a noticeable difference was observed between the enzyme-treated samples, the CTL and CTL+H_2O. The acid value and peroxide value in the CTL sample were higher than those in the enzyme-treated sample ($p<0.05$), while these values were highest in the CTL+H_2O sample. A study by Ma *et al.* (2022) also showed that the enzyme treatment positively affected the quality of macadamia oil when the acid value and peroxide value were lower than that of the solvent-based extraction method [23].

In addition, the quality of the oil was evaluated through sensory assessment. The results of the sensory quality assessment (Figure 5) showed no significant difference between the treatments in terms of color, flavor, taste, and overall evaluation. However, in the CTL+H_2O treatment, the oil's aroma received a lower assessment than the other samples. This finding is consistent with the earlier experimental results where the CTL+H_2O sample had the highest acid and peroxide value. Based on the analysis, it can be concluded that adding enzymes to the treatment of macadamia nuts before pressing does not significantly impact the physicochemical composition and overall quality of the macadamia oil product. As oil extraction efficiency tends to increase with the concentration of treated enzymes, it's worth noting that the color and flavor parameters maintain similar quality. However, when the enzyme concentration was increased to 0.6%, the results showed that, while the oil extraction efficiency increased (reaching 60.7%), this increase was not statistically significant compared to the 0.5% concentration (59.4 %).

A prior study by Picuric-Jovanovic *et al.* (1997), which involvedan aqueous-enzymatic extraction process of plum kernel oil, also showed that the maximum oil yield of approximately 70% was achieved at an enzyme concentration of 0.5% [24]. Therefore, considering the economic efficiency, the treatment of Viscozyme® L enzyme at a concentration of 0.5% was selected for further experiments.

Table 3. Quality parameters of macadamia oil

Targets	Viscozyme® L. enzyme concentration (%)					
	0.3%	0.4%	0.5%	0.6%	CTL	CTL+H_2O
Acid number (mgKOH/g)	$0.52^{AB}\pm0.01$	$0.51^{AB}\pm0.01$	$0.48^{A}\pm0.02$	$0.48^{A}\pm0.02$	$0.53^{B}\pm0.02$	$0.63^{C}\pm0.02$
Peroxide Value (meq/kg)	$1.34^{A}\pm0.03$	$1.33^{A}\pm0.03$	$1.29^{A}\pm0.03$	$1.28^{A}\pm0.03$	$1.57^{B}\pm0.04$	$2.57^{C}\pm0.04$

A, B, C, D: Represent values with statistically significant difference ($p<0.05$) in the same row.

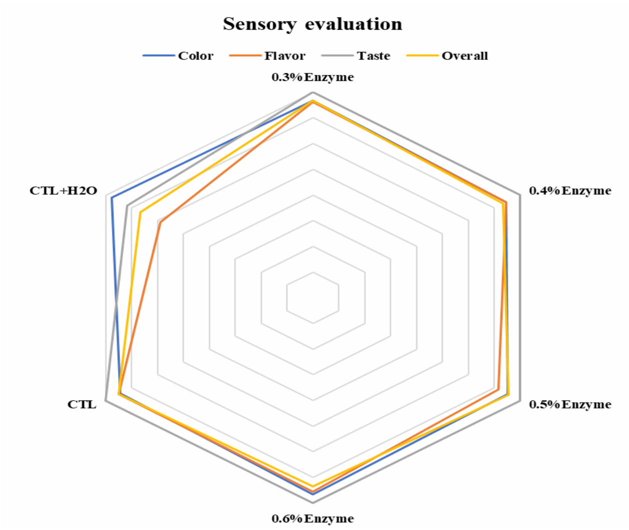

Figure 5. Sensory value of macadamia oil

3.3. Effect ofenzymetreatment time and added water ratio

In order to enhance the contact of the enzyme with the substrate, it is necessary to add water at the appropriate rate during the treatmentof the raw material, combined with the correct treatment duration. Macadamia kernel/nut material, after being ground to a size of <2mm, is soaked and treated with 0.5% Viscozyme® L enzyme for 3 different periods: 1 hour, 2 hours, or 3 hours, with water added at the rates of 20%, 30%, and 40% depending on the treatment (Table 4).

Table 4. Oil extraction efficiency (%) at different added water ratios and treatment time

Factor A (Processing Time)	Factor B (ratio of water/material)		
	B1 (20%)	B2 (30%)	B3 (40%)
A1 (1 hour)	54.4 ^{Ab} ± 0.85	58.3 ^{Ba} ± 0.73	60.0 ^{Ba} ± 0.77
A2 (2 hours)	55.3 ^{Ab} ± 1.32	65.2 ^{Aa} ± 0.78	64.7 ^{Aa} ± 0.86
A3 (3 hours)	54.9 ^{Ab} ± 1.40	66.0 ^{Aa} ± 0.65	63.9 ^{Aa} ± 0.84

A, B, C, D: Represent values with statistically significant differences ($p<0.05$) in the same column; a, b, c, d: represent values with statistically significant differences ($p<0.05$) in the same row.

The results presented in table 4 initially show that the enzyme treatment duration of 2 hours and 3 hours resulted in higher oil extraction efficiency compared to a 1-hr treatment. In the formulation with an additional 20% water content, longer treatment times they led to increased oil recovery efficiency. In particular, the 30% and 40% water additions during 2 hours or 3 hours of enzyme treatment yielded the highest oil recovery. The fact can explain this that adding sufficient water provides the enzyme with a better opportunity to contact the cells within the material, thereby supporting the hydrolysis reaction of substances surrounding the oil, ultimately facilitating the release of oil. Additionally, adequate water addition alters the physical properties of the nuts, making them more porous and better suited to support the oil pressing process. However, excessive water does not further enhance the oil extraction process; on the contrary, it increases the cost of the drying process and can lead to higher viscosity in the raw material fed into the press, consequently increasing the production cost of the machine and final product.

Table 5. Quality parameters of macadamia oil

physicochemical properties	Factor A (Time)	Factor B (Rate of added H_2O)			Mean
		B1 (20%)	B2 (30%)	B3 (40%)	
Acid value (mgKOH/g)	A1 (1 hour)	0.54 ± 0.04	0.52 ± 0.02	0.51 ± 0.02	0.52 ^{B} ± 0.03
	A2 (2 hours)	0.53 ± 0.01	0.47 ± 0.03	0.48 ± 0.02	0.49 ^{A} ± 0.03

	A3 (3 hours)	0.53 ± 0.03	0.48 ± 0.02	0.49 ± 0.03	0.50 ^{AB} ± 0.03
	Mean	0.53 ^{B} ± 0.02	0.49 ^{A} ± 0.03	0.50 ^{A} ± 0.02	
Peroxide value (meq/kg)	A1 (1 hour)	1.46 ±0.01	1.33 ±0.03	1.34 ±0.02	1.38 ^{B} ±0.06
	A2 (2 hours)	1.41 ±0.02	1.29 ±0.03	1.29 ±0.03	1.33 ^{A} ± 0.06
	A3 (3 hours)	1.42 ±0.02	1.30 ±0.01	1.32 ±0.01	1.34 ^{A} ±0.05
	Mean	1.43 ^{B}±0.02	1.31 ^{A}±0.03	1.32 ^{A}±0.03	

A, B, C, D: Represent values with statistically significant differences ($p<0.05$) in the same column or in the same row.

Similar to the previous findings, in order to evaluate how the quality of the obtained macadamia oil is influenced by the enzymatic treatment time and the percentage of added water, the acid value and the peroxide value were analyzed. As shown in table 5, the processing conditions, specifically the enzyme treatment time and the percentage of added water, had a slight impact on the quality of the macadamia oil, as reflected in the acid and peroxide values. When evaluating the effect of processing time in the enzyme treatment, oil obtained after pressing for 2-3 hours tended to have lower acid and peroxide values than oil treated for 1 hour. Furthermore, the use of 30% and 40% water in the enzyme treatment demonstrated a positive effect on the quality of macadamia oil after pressing, with lower acid value and peroxide value (*$p<0.05$*) compared to the formula with 20% water. An earlier study by Concha *et al.* (2004) had similar results, claiming that a water ratio of 30% gave a higher probability of oil extraction yield than treatment with a water ratio of 20% or 40% [25]. Based on these initial results, the combination of the addition of 30% water and treating macadamia with enzymes within 2 hours was chosen to complete the oil extraction process in the next stage.

3.4. Effect of pressing temperature

Macadamia nuts are highly sensitive to heat due to their rich content of unsaturated fatty acids, vitamins, and other biologically active compounds. In the oilpressing process, high temperatures can lead to oil denaturation, and other components such as glucid can easily be caramelized, causing the oil to darken. Additionally, oxidation may occur, adversely affecting the overall quality of the oil. In conventional hydraulic pressing method, the pressing temperature typically ranges from 30°C to 50°C. It's noting that at temperatures below 35°C, most of the characteristics related to the quality of macadamia oil are preserved. To evaluate the impact of temperature, the experimental process was conducted within the range of 25 to 40°C, meaning that all the quality characteristics of macadamia oil were maintained and better oil press efficiency.

The initial results in Table 6 showed that the oil extraction efficiency is higher when the oil is pressed at higher temperatures. At a temperature of 40°C, the highest oil extraction efficiency reached 70.7%, and this difference was statistically significant compared to the other conditions. At 30°C and 35°C, the oil extraction efficiencies were 66.8% and 68.1%, respectively, with no statistically significant difference (*$p>0.05$*). The lowest oil extraction efficiency was observed at 25°C. Therefore, in terms of oil recovery efficiency, a temperature of 40°C appears to be suitable for use in the cold pressing process. However, since temperature often impacts the quality of oil, it is essential to analyze the quality parameters and storage time when using a temperature of 40°C. To evaluate the quality of macadamia oil obtained and understand how is it affected by pressing temperature, we analyzed two important quality parameters of oils: acid value and peroxide value. The analysis results for the acid value and peroxide value are presented in table 7, showing that pressing temperature indeed affects both acid value and peroxide value. Specifically, as the temperature gradually increases, the acid value and peroxide value tend to increase, with the highest values observed in the pressed sample pressed at 40°C. Previous studies have also mentioned the changes and influence of pressing temperature on oil quality.

Table 6. Oil extraction efficiency (%) at different pressing temperatures

	Oil pressing temperature (°C)			
	25	30	35	40
Oil extraction efficiency (OY, %)	64.6 ^{C} ± 0.84	66.8 ^{B} ± 0.57	68.1 ^{B} ± 0.75	70.7 ^{A} ± 0.74

A, B, C, D: Represent values with statistically significant difference ($p<0.05$) in the same row.

Enzymes typically function within a specific temperature range. According to Rui *et al.* (2009), the optimum temperature range for enzymatic hydrolysis falls between 40 - 55°C. As a result, most studies are often performed within this temperature range [26]. In practical application, it is generally preferred to use the lowest possible temperature while still achieving adequate enzyme activity [27]. For instance, when dealing with olives, temperatures lower than 30°C are considered particularly favorable for oil preserving oil quality [28, 29, 30, 31]. Gros *et al.* (2003) also used a temperature of 34°C for similar reasons in the extraction of linseed oil [32]. Temperature plays a significant role in influencing oil yield, as demonstrated by Sharma *et al.* (2002), where the efficiency of peanut oil extraction was highest at 40°C and significantly decreased when the temperature was lowered to 37°C [33]. According to Zuniga *et al.* (2003), temperatures exceeding 45°C led to a decreasein the enzyme hydrolysis due to enzyme inactivation, resulting in lower oil yield [34].

In addition, the presence of soluble sugar in the mixture can limit oil release from the cell and cause caramelization during drying. As a result, oil output generally increases up to a certain temperature, after which it stabilizes or gradually decreases. This trend is observed in the majority of studies. Therefore, when selecting an enzyme treatment temperature, both oil yield and oil quality characteristics must be taken into account.

Table 7. Macadamia oil quality parameters

Targets	Oil pressing temperature (°C)			
	25	30	35	40
Acid value (mgKOH/g)	$0.51^{A} \pm 0.02$	$0.53^{A} \pm 0.04$	$0.76^{B} \pm 0.05$	$1.34^{C} \pm 0.08$
Peroxide value (meq/kg)	$1.29^{A} \pm 0.05$	$1.32^{A} \pm 0.03$	$1.98^{B} \pm 0.07$	$3.71^{C} \pm 0.06$

A, B, C, D: Represent values with statistically significant difference ($p<0.05$) in the same row.

It has been well known that fatty acids are recognized as the primary components of macadamia oil, and they play an important role in determining the quality of macadamia oil. In order to have a better understanding of how diferent treatment methods influence the quality of macadamia oil, we analyzed the fatty acid composition of macadamia oil. The analytical results presented in table 8 showed that the fatty acid composition in macadamia oil primarily consists of unsaturated fatty acids, accounting for > 80% of the total fatty acids. Specifically, the fatty acids found in large amounts in macadamia oil include oleic acid (52~55%), palmitoleic acid (20.5~24%), palmitic acid (10.3~11.8%), stearic acid (2.54~5.53%), linoleic acid (1.92~3.13%).

Among the unsaturated fatty acids, oleic was the most abundant, while among the saturated fatty acids, palmitic acid predominant. These results in our study are similar with those of previous studies when analyzing the fatty acid composition in macadamia oil. For example, Aquino-Bolaños *et al.* (2017) analyzed 20 fatty acids and found that the most common ones in macadamia nut oils were oleic acid (40 - 51%), palmitoleic acid (24 - 36%)) and palmitic acid (8.4 - 13.1%) [35]. Akhtar *et al.* (2006) also reported that unsaturated fatty acids in macadamia oil accounted for 80 - 84%, with the 03 acids present in the highest content content being oleic acid (54 - 68%), palmitoleic acid (16 - 23%) and palmitic acid (7 - 10%) [36]. Recently, Shuai *et al.* (2021) analyzed the acid composition in 15 macadamia varieties harvested in China and obtained similar results. Specifically, the unsaturated fatty acid composition in macadamia oil ranged from 82.8 - 85.9%, while saturated fatty acids accounted for 14.1 - 17.2%. Furthermore, there was no significant difference in fatty acid composition among these varieties [37]. In another study conducted by Shuai *et al.* (2022), it was similarly shown that the fatty acid composition of macadamia oil did not significantly vary based on different extraction methods [2]. The results of the present analysis in this experiment also showed that there was no major difference in the fatty acid composition of the samples treated under various conditions. It is evidence that the enzyme method offers advantages such as higher oil recovery efficiency and consistent oil quality. The results of this experiment further reinforce the fact that macadamia oil contains a high content of unsaturated fatty acids (constituting more than 80% of the total fatty acid content in the oil), while the percentage of saturated fatty acids is low (<15%). This information reaffirms the quality and health benefits of macadamia nut oil.

Table 8. Fatty acid composition and some quality values in macadamia nut oil

Fatty acid composition (g/100 g)	Enzyme	CTL+H_2O	CTL
Butyric acid	0.068	0.039	0.054
Caproic acid	0.075	0.061	0.098
Caprylic acid	0.077	0.085	0.091
Capric acid	0.057	0.174	0.059
Undecylic acid	0.025	0.038	0.057
Lauric acid	0.081	0.091	0.084
Myristic acid	0.815	0.83	0.726
Methyl Cis-10-Pentadecanoate	0.046	0.086	0.048
Palmitic acid	10.8	11.0	10.3
Palmitoleic acid	24.0	20.5	21.1
Stearic acid	2.54	3.25	5.53
Oleic acid	55.0	53.1	54.4
Linoleic acid	2.13	2.07	1.92
α-Linolenic acid	0.11	0.11	0.12
Arachidic acid	Nd	4.63	0.23
cis-11-Eicosenoic acid	1.44	1.44	1.35
Behenic acid	2.52	1.29	2.56
Erucic acid	0.079	1.15	1.26

Nd: Not detected (LOD=0.008)

4. CONCLUSION

In this study, we evaluated the impact of using enzymes in the pretreatment stage on the pressing performance and some quality parameters of the resulting macadamia nut oil. Out of the three enzymes investigated, Viscozyme® L was selected as the most effective. When combined with Viscozyme® L and a crushed particle size less than 2 mm, with the 0.5% enzyme concentration, the highest oil recovery efficiency reached 59.4%, which is approximately 20% higher than the control samples without enzymes. When using a 0.5% enzyme concentration in combination with 30% or 40% added water during a 2 hours or 3 hours treatment, the highest oil extraction efficiency was achieved. The optimal temperature achieving good oil pressing results was found to be 30^{o}C, where the oil extraction efficiency reached 66.8%. Quality parameter of the oil, including the acid value, peroxide value, fatty acid composition, and others, were analyzed, demonstrating that the oil met the quality standards expected of vegetable oils. Our current research has demonstrated that enzyme treatment has a positive impact on increasing macadamia oil yield while maintaining its quality characteristics. However, before considering large-scale implementing to replace traditional method with enzymes treatment, it is imperative to conduct a thorough economic assessment.

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CHILLING UNIT IN RELATION TO THE DEVELOPMENT OF TEMPERATE FRUITS IN THE NORTHERN MOUNTAINOUS PROVINCE OF VIETNAM

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ABSTRACT

Aimed to properly determine the impact of weather condition in global climate changes circumstance specialized in annual chilling unit (CU) to the temperate fruit development in the Northern mountainous provinces, a study in the form of basically over all investigation was systematically conducted in which the method of overlapping the suitably related maps, the technologies of participatory rural appraisal (PRA), key informative persons (KIP) and geographical information system (GIS) were used as the principal tools. Results conducted from the study showed that the annual chilling unit calculated in two recent decades was unremarkably changed, closely related to the distribution of temperate fruit production in the studied region and it can be necessarily used for planning and projecting the development of these crops in the Northern mountainous provinces.

Keywords: *Investigation, temperate fruit, chilling unit, the Northern mountainous of Vietnam.*

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1. INTRODUCTION

Temperate fruit development in Vietnam is a step in the right direction, especially in Northern mountainous provinces that account for 30% of the total area but only contribute 9.6% of the total GDP. In addition to helping implement poverty alleviation and support the lives of people in these difficult areas, this development is to ensure food safety and reduce the amount of imported produce (especially from China). In this sense, temperate fruit production indirectly contributes to Vietnam's competitively advantaged fruit exports.

Temperate fruit in general and varieties of persimmons, pears, peaches, and plums in particular require a certain period of time in a year with chilling temperature suitable for floral initiation and development. Such fruit crops are mostly distributed in high-latitude countries of the world, mainly in Europe, America, and Northeast Asia. In fact, most temperate fruit cultivars need a high chilling requirement. For example, peach cultivars require about 600 - 1000 chilling units (CU), whereas plum cultivars require around 800 – 1200 CU [1]. Therefore, advances in fruit varieties and farming techniques are hard to be adapted to mountainous regions with subtropical climates in Southeast Asia, including Vietnam.

The sufficient accumulation of chilling required for floral bud initiation is in the genetics of the variety. In general, the majority of temperate fruit varieties require a temperature threshold for flower initiation between 0^0C and 15^0C, as a result of which, under temperature conditions below 0^0C or above 15^0C, flower bud formation cannot occur [2]. There is a growing demand for temperate fruit in Vietnam, particularly in big cities and places where the population is concentrated. High mountainous areas in the north and the central highlands with cold winters and receiving sufficient chilling are suitable for growing low-chill, temperate fruits. Varieties requiring medium-chill units can be grown in some locations. Ethnic

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minorities traditionally grow temperate fruits at an altitude of 800 - 2000 m above sea level [3]. Moc Chau district is largely situated on a plateau at an elevation between 800 and 1300 m above sea level, and with 200 - 600 chill units (CU), it is the most suitable district for temperate fruit production in Son La province. Lao Cai province has several districts, including Bac Ha, Sa Pa, Si Ma Cai, Bat Xat, and Muong Khuong, situated on high elevations above 1000 m having a cool climate with CU from 250 - 550 [4].

Using the Utah model or Dynamic and George - Nissen's simplified formula, which is later built into a specialized software, the number of chilling units (CU) of a region can be calculated, making the use of native or introduced varieties proactive and effective. This is also the reason why chilling unit have been studied over the last two decades in relation to the distribution and potential for temperate fruit development in the Northern mountainous regions of Vietnam.

2. RESEARCH METHODS

2.1. Studied objective

Chilling unit and current status of temperate fruit trees (pear, plum, peach, and persimmon) development in the northern mountainous provinces North Vietnam.

2.2. Methodology

Setting up questionnaires: Set up the questionnaires to gather information related to natural and social conditions in the provinces capable of temperate development (chilling unit – CU of 50 or more) in the Northern mountainous areas. These conditions include land, climate, weather, population, labor, traffic, etc. and the current condition of fruit tree production, especially temperate fruit tree production (area, variety, growth, fruit development)

Selecting locations for the survey: The overall investigation was conducted in Northern mountainous provinces to gain an overview of the current situation and calculate the possibility of developing temperate fruit trees throughout the region. Those regions with traditional development as well as sub-regions that have competitive advantages and are highly representative of each eco-sub-region should be a priority.

Carrying out the investigation: The application of participatory rural appraisal (PRA) methodology and key informative persons (KIP) method was used to gather primary data and information from relevant organizations (research institutes, universities, the Provincial Department of Agriculture and Rural Development, agriculture divisions etc.) along with undertaking fieldwork to assess the current condition of temperate fruit tree development in Northern Vietnam.

Calculating chilling unit (CU): By using the Prochill software, the average low temperature parameters of the coldest months of the year (mainly December, January and February depending on the year) were used to calculate the CU, referenced and compared with the Utah model (deduced from the temperature in 1 hour) and George – Nissen formula: ($Y = a+b/x^d$)

Processing survey results and planning development: Based on the climatic requirements of temperate fruit varieties, the GIS (geographical information system) method were utilized along with the use of specialized software like Mapinfo 10.5, ARCGIS 10 to overlap all kinds of adaptation maps (on land, climate, infrastructure, consumption market), in which adaptation maps on climate were the main focus.

2.3. Time and place of study

The study was carried out during the whole year of 2019 in some mountainous provinces in the North of Vietnam

3. RESULTS AND DISCUSSION

3.1. Chilling unit in recent years

Table 1. Synthesis of chilling unit accumulation (CU) in some areas
(Using Prochill software, referred to George – Nissen formula: $Y = a+b/x^d$)

Location	2001	2002	2003	2004	2005	2006	2007	2008	2009	Average
Moc Chau district	167.1	303.1	350.8	253.0	280.6	220.9	340.2	316.3	395.0	*291.9 (5)*
Bac Yen district	82.2	198.8	221.0	142.1	196.0	134.8	212.5	207.0	253.0	183

Phu Yen district	-15.9	75.8	95.4	40.6	84.4	22.9	86.6	104.4	135.0	69.9
Co Noi district	48.6	185.3	180.1	106.7	144.6	123.0	165.0	169.7	253.0	152.9
Sin Ho district	309.7	650.7	463.7	430.5	406.6	398.9	503.4	410.5	588.8	*462.5 (2)*
Tam Duong district	134.8	250.0	214.5	148.8	265.1	185.3	229.5	185.3	296.6	*211.9 (7)*
Sa Pa town	485.5	662.1	741.4	628.3	633.9	601.4	974.0	650.7	915.0	*699.1 (1)*
Bac Ha district	259.0	336.8	379.9	309.7	383.7	309.7	410.5	446.9	508.1	*371.6 (3)*
Yen Bai province	28.7	99.9	120.6	118.2	142.1	56.8	140.0	188.0	177.4	119.1
Luc Yen district	21.0	99.9	113.6	99.9	137.2	73.6	152.0	185.3	191.0	119.3
Mu Cang Chai district	127.1	223.8	235.3	204.2	241.1	182.7	198.8	177.4	383.7	*219.3 (7)*
Nghia Lo town	22.7	99.9	120.6	80.1	120.6	67.3	130.0	159.5	180.1	109
Ham Yen district	28.6	82.2	109.0	95.4	132.4	54.8	125.3	174.8	190.7	110.4
Ha Giang province	44.6	86.6	111.3	99.9	147.0	67.3	157.0	174.8	198.8	120.8
Bac Quang district	26.8	93.2	111.3	95.4	120.6	58.9	139.7	174.8	177.4	110.9
Cao Bang province	113.6	172.3	111.3	142.1	259.0	95.4	244.3	340.2	256.0	192.7
Trung Khanh district	212.5	293.3	347.2	323.1	351.2	244.3	402.7	575.5	395.3	*349.5 (4)*
Bac Kan province	61.0	113.6	157.0	137.2	172.3	84.4	170.0	232.4	215.3	149.2
Ngan Son district	182.7	259.0	253.0	280.6	233.3	190.7	350.8	451.1	402.7	*289.3 (5)*
Bao Lac district	154.7	118.2	149.5	104.4	159.5	75.8	193.3	209.7	212.5	152.9
Dinh Hoa district	36.6	97.7	147.0	120.6	152.0	56.9	147.0	207.0	196.4	129
Lang Son province	152.0	209.2	241.1	226.6	303.1	165.0	271.2	442.7	313.0	*258.2 (6)*
That Khe town	123.0	164.6	221.0	188.0	274.3	128.0	247.8	383.7	265.1	*221,7 (7)*
Son Dong district	146.6	99.9	95.4	84.4	137.2	73.6	113.6	209.7	177.4	126,4
Luc Ngan district	34.6	93.2	139.7	113.3	149.5	58.9	125.3	232.4	177.4	124.9

Tien Yen district	73.6	123.0	169.7	127.7	169.7	84.4	149.5	215.3	182.7	144
Da Lat city	77.9	102.2	95.4	-55.0	109.0	67.2	75.8	77.9	147.0	77.5
Bao Loc district	-60.0	-59.9	-59.9	-38.4	-48.5	-72.6	-58.3	-80.4	-58.3	-59.6

In the context of accelerating climate change with global temperature increasing over time, the first issue that needs addressing is to consider whether traditional highlands in Vietnam in general and the northern mountainous areas in particular can meet mandatory demand for sufficient chilling. If the demand can no longer be met, what solutions are needed to maintain the available temperate orchards.

Meteorological data were collected in these regions, especially the minimum average temperature in each area. Some of the regions are representatives of the Northern mountainous areas, where temperate fruit cultivation is exploited such as Moc Chau district (Son La province), Dong Van district (Ha Giang province), Trang Dinh district (Lang Son province), Bac Ha district, Sa Pa town (Lao Cai province).

After the calculation using Prochill software along with references to the Utah model and formula proposed by George *et al.* (1998b) [5], the data is presented in graphs and divided into two stages: from 2001 to 2009 (Table 1 and figure 1a, 1b) and the most recent 5 years from 2015 to 2019 (Figure 2).

Comments:

* From 2001 to 2009: The CU value for 10 years in each location changed over time but generally there was no major fluctuation except for Sa Pa town in 2002 and Sin Ho district in 2007, 2008, and 2009. This means that in general, the accumulation of chilling is relatively stable in the context of climate change.

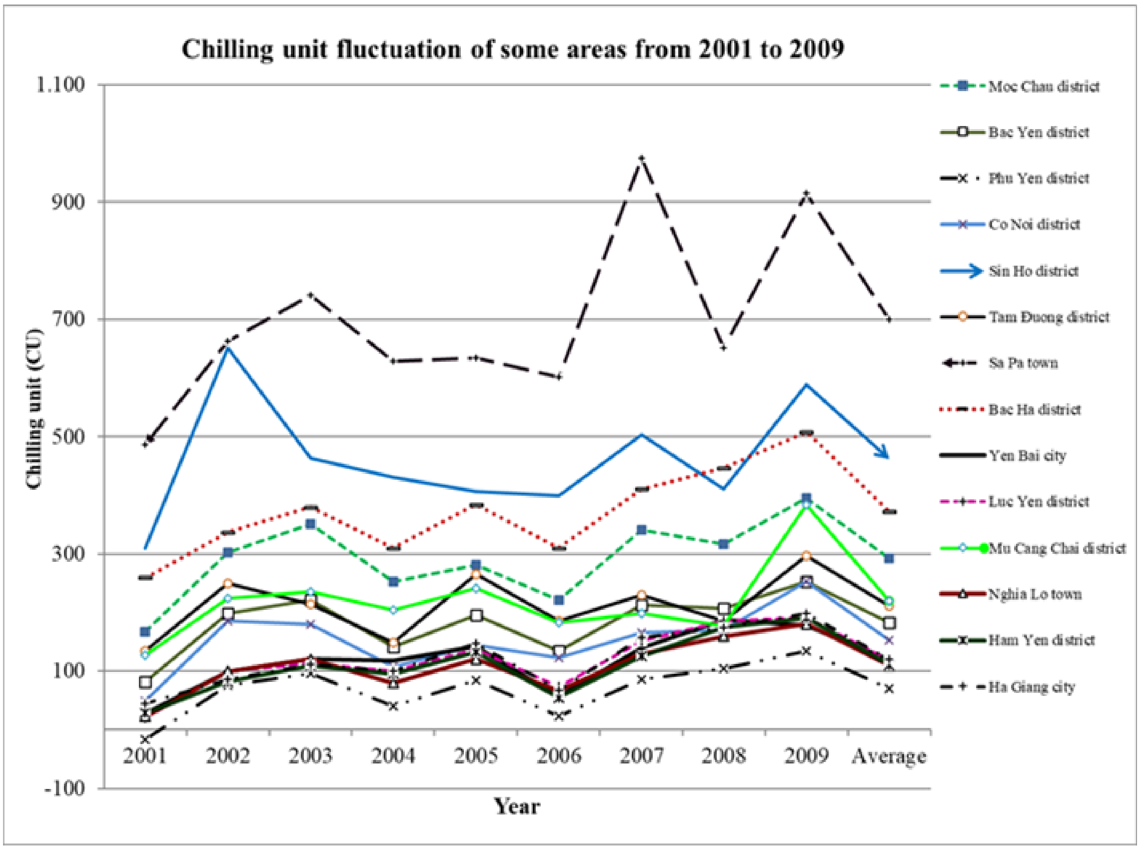

Figure 1a. Chilling unit fluctuation of some areas from 2001 to 2009

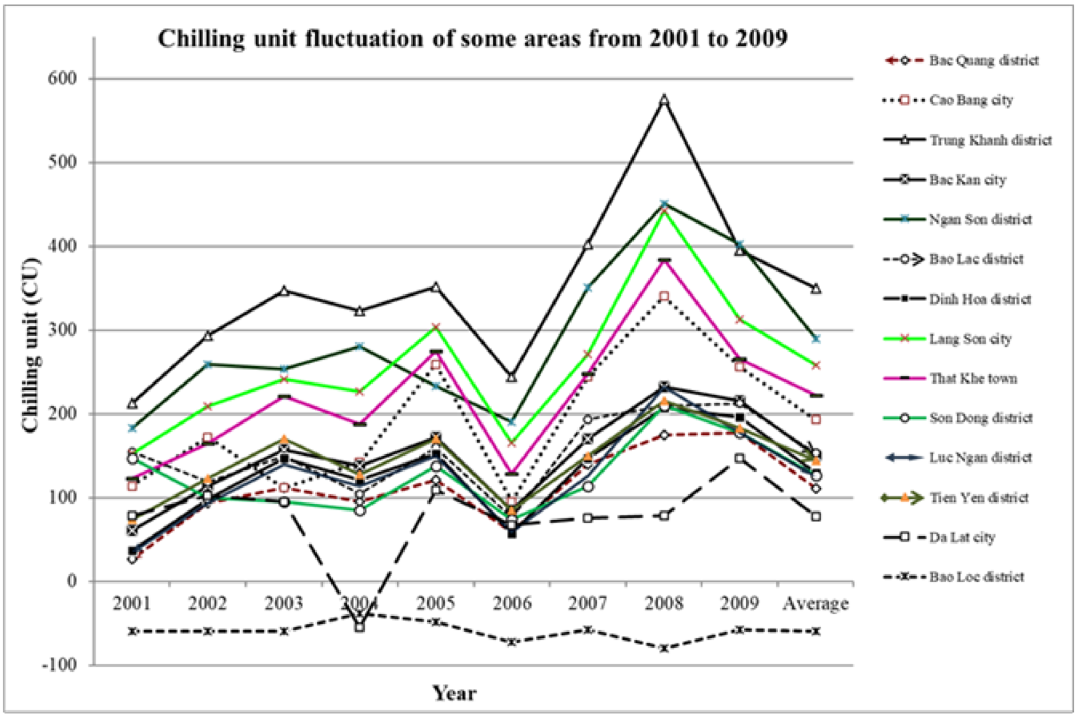

Figure 1b. Chilling unit fluctuation of some areas from 2001 to 2009

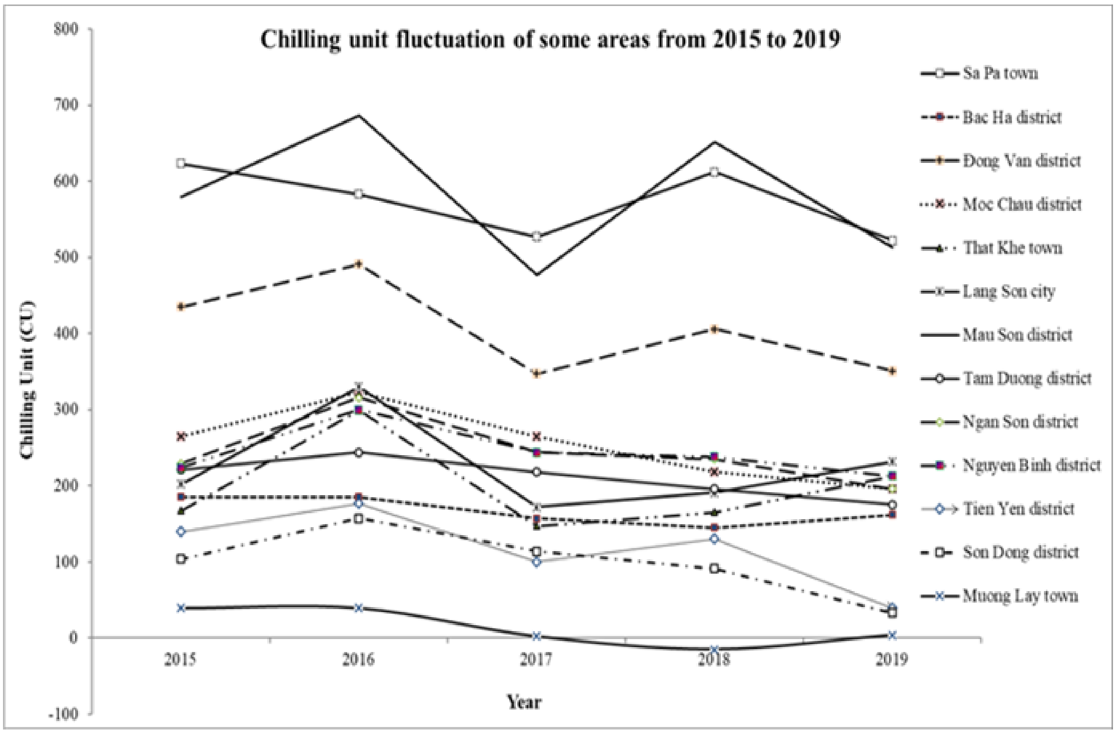

Figure 2. Chilling unit fluctuation of some areas from 2015 to 2019

Based on the average number of chilling hours in 10 years (ranked in parentheses attached to the following) and compared to the chilling requirement of temperate fruit varieties in general, the studied areas can be divided into 3 groups:

- Group 1: Includes Sa Pa town and Sin Ho district with chilling hours between 450 and 650 CU, especially with a few years exceeding 700 CU. This value range allows floral initiation for temperate fruit varieties with high chilling requirement (high chill cultivars).

- Group 2: Includes Bac Ha district, Trung Khanh district, Moc Chau district, Ngan Son district, Tam Duong district and That Khe town with the average number of chilling hours ranging from over 200 CU to below 400 CU, mainly between 200 and

300 CU. This chilling range allows cultivation of moderate chill cultivars and low chill cultivars.

- Group 3: Include the remaining areas with chilling hours around 100 CU, even with negative values (such as Bao Loc district, Lam Dong province). Temperate fruit production and development is not recommended in these areas. Only places with chilling hours of approximately 100 CU (such as Son Dong district and Tien Yen district) can be considered, provided that these areas are given varieties with low chilling requirement and supported with technical measures (leaf cutting, branch downwards; growth regulator application.

* From 2015 to 2019: With the aim of making a feasible plan for developing temperate fruit, the research focused only on regions with more advantages in chilling accumulation. Dong Van district, which represents 4 upland districts of Ha Giang province, is added as a sample to the study. This area has high potential due to suitable climatic conditions for growing temperate fruit trees and in practice, its contribution to the local economy is significant (Table 1).

The fluctuation of the chilling unit tends to be similar to that in phase 1. Overall, the annual number of chilling hours fluctuates slightly, but the fluctuation is not significant. These studied regions basically ensure the flower initiation of moderate and low chill cultivars, in which the low chill varieties need to be paid more attention.

3.2. Development of temperate fruit trees in some Northern mountainous provinces in recent years

Aimed to understand the development of temperate fruit trees in relation to climatic conditions, several locations that had sufficient chilling accumulation for flower initiation of some temperate fruit varieties were selected for sampling. The figures were summarized in table 1, 2 and 3.

Table 2. Area and production of temperate fruit trees in Ha Giang province

No	Species	2016		2017		2018		2019	
		Area (ha)	Total yield (tons)	Area (ha)	Total yield (tons)	Area (ha)	Total yield (tons)	Area (ha)	Total yield (tons)
1	Pear	724.0	2,103.8	946.5	2,300.5	980.5	2,517.9	1,282.7	4,966.6
2	Plum, peach	1,148.9	1,973.8	1,147.9	1,839.4	1,183.8	2,044.0	1,920.1	4,561.5
3	Persimmon					445.9	523.2		

In all 3 locations, the area and total output tended to increase significantly in recent years, especially in Ha Giang province (Table 1) and districts such as Moc Chau district (Table 2) and Bac Ha district (Table 3), in which production of pear trees in all 3 locations, plum trees and peach trees in Ha Giang province has increased quite considerably in the last 3 or 4 years.

On the whole, the increase in area that entails an increase in the production of temperate fruit trees was due to various subjective and objective causes. The temperature factor, namely the chilling unit accumulation, was not a sufficient condition yet it was a necessary and mandatory factor. The data presented in the tables of this article is only intended to add a testament to the fact that climate change has not had a considerable impact on chilling accumulation. Some varieties of temperate fruit trees can still flower and bear fruit despite rising global temperature.

It should also be added that the chilling unit (CU) value is not equivalent to coldness. The chilling unit is determined at a certain period of time in the rotation of a year when temperate plants in general and temperate fruit trees in particular move from vegetative staging to reproductive staging, precisely the preinitiation stage.

Table 3. Area and production of temperate fruit trees in Moc Chau district

No	Species	2016		2017		2018	
		Area (ha)	Total yield (tons)	Area (ha)	Total yield (tons)	Area (ha)	Total yield (tons)
1	Plum	1,413.0	13,273.0	1,439.0	11,299.0	1,951.0	16,700.0
2	Persimmon	19.0	465.0	41.0	442.0	45.0	464.0
3	Peach	42.0	175.0	48.0	184.0	58.0	197.0
4	Pear	133.0	822.0	115.0	693.0	105.0	499.0
	Sum	1,607.0	14,735.0	1,643.0	12,618.0	2,159.0	17,860.0

Table 4. Area of some temperate fruit trees in Bac Ha district, Lao Cai province

Year	Area of land (ha)	Area of temperate fruit trees (ha)	% in area of land	Temperate fruit species				
				Peach	Persimmon	Pear	Plum	Others
2010	-	685	-	60	23	95	477	30
2011	-	840	-	80	23	135	572	30
2012	-	916	-	93	23	185	585	30
2013	-	983	-	98	23	247	585	30
2014	-	1,008	-	98	23	272	585	30
2015	-	1,035	-	98	23	286	593	35
2016	-	1,042	-	98	23	286	600	35
2017	-	1,092	-	105	23	286	643	35
2018	-	1,167	-	115	23	300	693	36
2019	4,6126	1,207	67,5	120	23	315	712	37

3.3. Chilling unit in relation to the issue of distribution and planning of temperate fruit cultivation

The map of the distribution of temperate fruit trees in the Northern mountainous region, as shown in Figure 1 is made using the results of the survey conducted in 2019 and 2020, referencing documents from local management agencies.

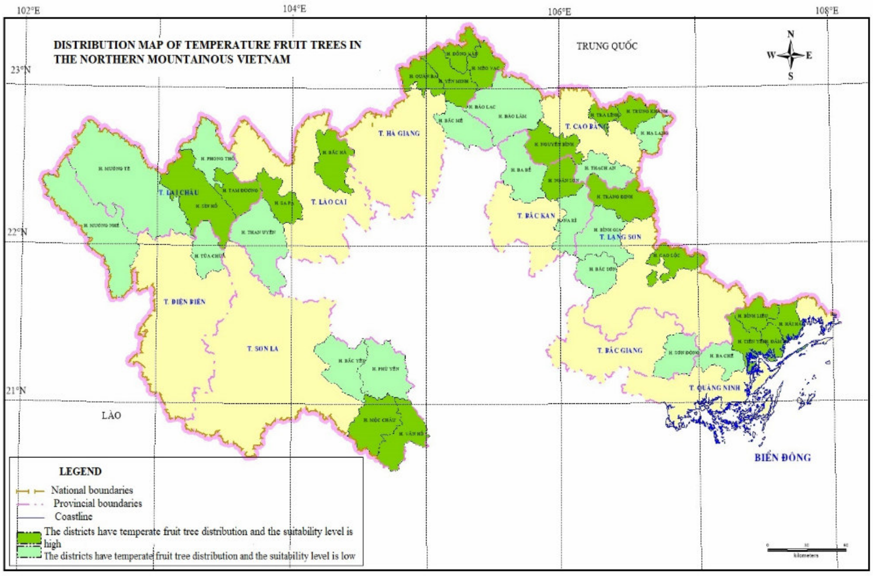

Figure 3. Map of the distribution of temperate fruit trees in the Northern mountainous region

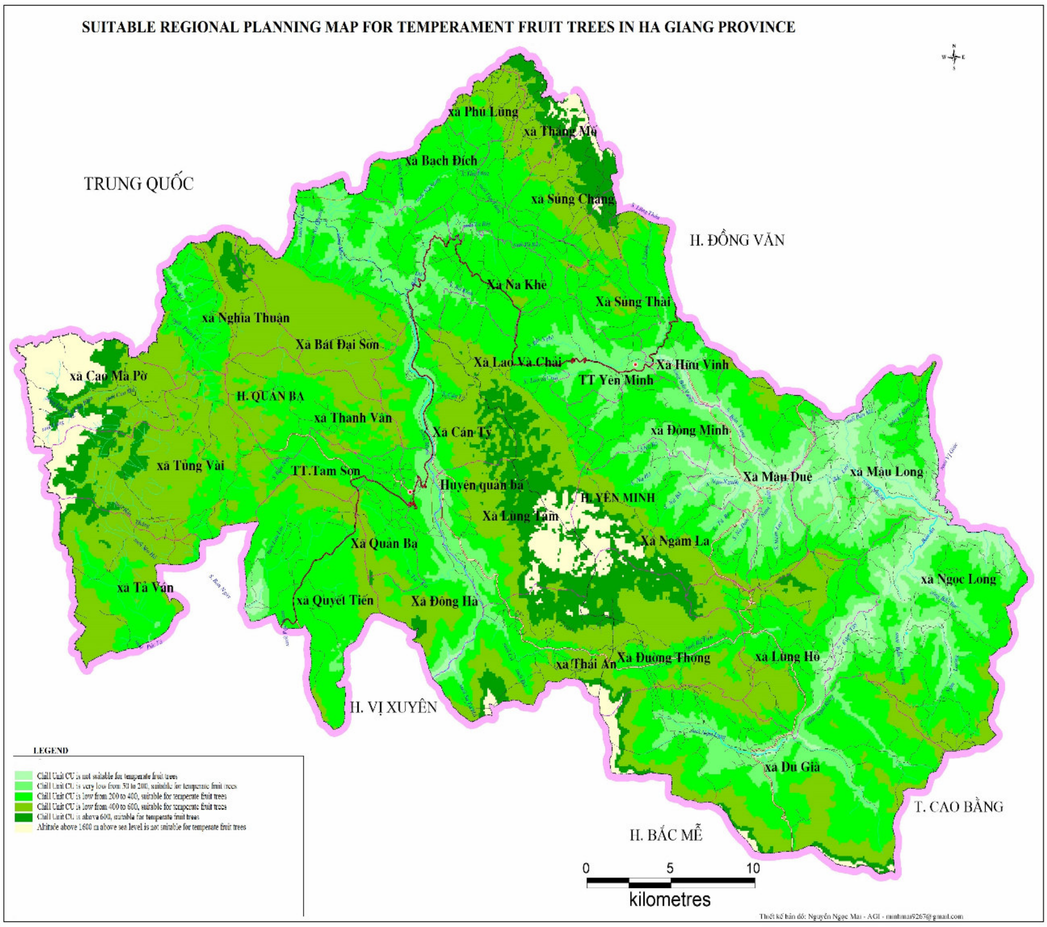

Figure 4. Map of temperate fruit tree planning in Ha Giang province

Planning maps are created for each region based on the average value of chilling unit (CU) over many years along with other climatic factors such as air humidity, monthly precipitation, radiation, while taking extreme factors into consideration like hoarfrost and hail. It also refers to and integrates information using GIS tools taken from land plots (soil type, thickness, steepness, components), using the overlapping maps method. Therefore, these maps are heavily inclined to the potential meaning of it. One of the main factors is the chilling unit value. An example is Ha Giang province (Figure 2) and 4 of its upland districts bordering China: Quan Ba district, Yen Minh district, Meo Vac district, and Dong Van district with high chilling accumulation (above 500 CU), which shows great potential for temperate fruit development.

Thus, it can be indicated that the chilling unit is closely related to the current distribution/development and planning of planting areas for temperate fruit trees. Due to the limitations within the framework of an article, this will be analyzed further on another occasion.

4. CONCLUSION

The chilling unit value in most Northern mountainous provinces maintains a constant relative stability despite abnormal changes in the weather conditions.

In regions with potential climatic conditions, temperate fruit trees tend to grow quite strongly in recent years.

Chilling unit value is closely linked to the current state of distribution and is one of the basic factors in elaborating the planning on temperate fruit development in the Northern mountainous areas.

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ESTABLISHING THE TECHNICAL ADVANCES APPLIED DEMONSTRATION OF SERICULTURE PRODUCTION IN YEN BAI PROVINCE

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ABSTRACT

Yen Bai province is considered as one of the few locations with largely concentrated areas under sericulture cultivation Sericulture production has provided abundant jobs and opportunities for local residents, improved the lives of many households and reduced poverty in the area. The application of technological advances through large scale demonstrations is proved to be the best way for improving the economic efficiency of sericulture. In the duration of three years (2020 - 2022), 09 sericulture demonstrations covering a total area of 197.4 ha under mulberry cultivation have been established and developedwith the participation of 370 local households, producing 11,562 silkworm egg cards in Van Chan and Tran Yen districts, Yen Bai province. Results conducted from the study showed that these demonstrations made a positive impact to the production of mulberry and silkworm as indicated by the increase of mulberry leaf and cocoon yield (21.89% and 13% higher than normal, respectively). In addition, the cocoon harvesting time reduced to 67.19%, the rate of good cocoon increased by 7.19%. 100% of the sericulture farmers are using wooden square mountages.

Keywords: *Sericulture, mulberry, silkworm, Yen Bai.*

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1. INTRODUCTION

Sericulture is regarded as one of the long traditional sectors in agricultural production of Vietnam. Nowadays, though various kinds of synthetic fibers have been produced with the rapid development of chemical industry, silk and silk used products are highly appreciated by the consumers because of its unique characteristics. The production of sericulture chas become the main source of livelihood for 38,076 farming households and 101,705 sericulturists in 35/64 provinces across Vietnam, leading toa significant contribution to the socio-economic development in rural areas [1].

Sericulture has been developed in Yen Bai province since the 2000s. Over 20 years of trial and production in both small and large scale, sericulture was recognized and evaluated to have a significant contribution to the development of the provincial agricultural economy. It is obvious that sericulture production played an important role in the developing of the agricultural sector of Yen Bai province. At present, 1,090 ha of land under mulberry cultivation is , mainly concentrated in 3 districts named Tran Yen, Van Chan and Van Yen. The expanded land area of 1,500 ha for mulberry production up to 2025 was also oriented by local authority in accordance with properly supported policies proposed from local authority [2].

However, the sericulture production in Yen Bai province has faced a lot of challenges. Lower yield (30 - 32 tons of mulberry/ha/year; 1 - 1.1 tons of cocoon/ha on average) and unsustainable cultivating system are some of the obstacles that need to be addressed and solved accordingly. Hand made activities have been popularly applied in almost every steps of sericulture production from mulberry planting to cocoon harvesting, and this method was already regarded to be out of date. Most of the cultivation stagessuch as mulberry planting, grass cleaning, fertilizer application, silkworm feeding, cocoon harvesting are done by hand that require a lot of labor as compared to advanced methods applied in

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developed sericulture countries i.e., China, India, Korea, etc. The main issues of the sericulture production system in Yen Bai province. Included small scale of production, low yield, low productivity, resulting in low income, lack of value chain linkage, investment and policy.

It is, therefore, necessary to apply newly introduced variety of mulberry and silkworm, combined with technological advances to improve the yield and quality of mulberry leaves, silkworm cocoon, thus improve the local farmer's livelihood in Yen Bai province.

2. MATERIALS AND METHODS

2.1. Materials

- Mulberry: F1 hybrid mulberry variety GQ2.

- Silkworm: Double cross silkworm variety BT1218 and double cross silkworm variety VH2020.

- Mountage: 03 types: 1. W bamboo mountage; 2. Wooden square mountage (25 mm x 45 mm, have 21 x 37 dimensions rectangles); 3. Withdraw bamboo mountage

2.2. Methods

- **Methods of mulberry cultivation** in accordance with the Agricultural Standard No. 10TCVN 489-2001: the technical procedure for planting, caring and harvesting of hybrid variety of mulberry, issued by the Ministry of Agriculture and Rural Development presented in Decision No. 121/2001/QD/BNN dated 21st November, 2001 [3].

- **Young age silkworm rearing method:** Approved by the Ministry of Science as improved technology by Decision No. 1415/QD-BKHCN dated 18th August, 2004 [4].

- **Late age silkworm rearing method on the floor**: Approved by the Ministry of Science and Technology presented in Decision No. 2243/QD-BKHCN dated 26th July, 2011 [5].

- **Effect of mountage types used for silkworm to release silk on yield, quality of cocoons and silk:** Field trials of 3 treatments were accordingly designed:

Treatment 1: Type of W bamboo mountage was used.

Treatment 2: Type of wooden square mountage was used.

Treatment 3: Type of withdraw bamboo mountage was used (control).

When spinning larvae, 2500 silkworms were randomly selected and fed on the mountages. After 4 days, cocoons were removed from the mountages for research.

** Methods to investigate the data and information:*

- Based on the Standard TCVN 1697 - 87 and Chinese Raw Silk Standard GB 1797 - 2001 (National Textile Industry Bureau, 2001) [6, 7]

- Followed "National technical regulation on procedure of experiment, testing breeding silkworms" - QCVN 01 - 74: 2011/BNNPTNT. Evaluation traits: cocoon yield (kg/6 gr eggs), cocoon weight, cocoon shell weight, cocoon shell percentage, average filament length, denier, rendital, reelability [8]

** Data analysis:* Analysis of variance (ANOVA) was performed using IRRISTAR 5.0

To conduct the field trials, Vietnam Sericulture Research Center (Vietseri) closely cooperated with the Agriculture Department of Yen Bai province and farmers of Van Chan and Tran Yen districts.

3. RESULTS AND DISCUSSIONS

3.1. The impact of newly introduced demonstrations to the growth of GQ2 mulberry variety

Table 1. Quantity of mulberry seeds sown and seedlings in 2020 - 2022

No.	Sowing time	Mulberry seed (kg)	Total harvest (seedling)	Standard seedling	Standard seedling rate (%)
1	2020	12	1,397,600	1,275,000	91.23
2	2021	25	3,137,000	2,995,000	95.47
3	2022	32	3,431,000	3,257,000	94.93
	Total	69	7,965,600	7,527,000	

Mulberry seeds were sown and taken care of in the nursery located in Kinh Mon district, Hai Duong province. About more than 60 days after sowing, seedlings of ≥ 0.40 m length considered as standard ones were provided to farmers of Yen Bai province. Of the total 69 kg of mulberry seeds sown in the nursery, more than 91% seedlings were evaluated as standard ones (Table 1).

From the mentioned seedling quantity, 8 demonstrations of newly introduced mulberry variety coded GQ2 were established in Van Chan and Tran Yen districts during the year 2020 – 2022 that covered an area of 194.7 ha in total with the participation of 370 households (Table 2).

Table 2. Areas under mulberry cultivation in the demonstrations in 2020 - 2022

No.	Locations	Year	Area (ha)	Number of farmer household participated
1	Van Chan district (Son Thinh commune, Chan Thinh commune and Dong Khe commune)	2020	30	65
		2021	40	60
		2022	11.4	39
Sub- total			*81.4*	*164*
2	Tran Yen district (Y Can, Viet Thanh, Nga Quan, Quy Mong and Hoa Cuong)	2020	-	-
		2021	52	60
		2022	64	146
Sub- total			*116*	*206*
Total			197.4	370

Notes: (-) The project was started in Tran Yen district in 2021.

The growth of GQ2 mulberry variety in the demonstrations in Chan Thinh and Son Thinh communes of Van Chan district and Viet Thanh commune of Tran Yen district presented in table 3 showed that the variety is healthy in all 3 locations tested, as indicated by high survival, bud growing rates and big sized leaves.

Table 3. The growth of GQ2 variety in 3 demonstrations

Locations	Seedling survival rate (%)	Bud growing rate (%)	The length of tree (m)	Leaf size (cm)	
				Length	Width
Chan Thinh commune, Van Chan district	93.40	15.20	2.50	20.18	17.42
Son Thinh commune, Van Chan district	91.28	12.94	2.37	19.45	16.74
Viet Thanh commune, Tran Yen district	94.28	13.07	2.34	19.75	16.92
Average	92.99	13.74	2.40	19.79	17.03

When compared to Chinese variety, results conducted from the trials showed in table 4 stated that the leaf yield of GQ2 variety (41.91 ton/ha/year in average) were much higher than the Chinese one (32.74 ton/ha/year)

Table 4. Leaf yield of mulberry GQ2 variety planted in 2021

No.	Locations	GQ2 variety (ton/ha/year)	Chinese variety (ton/ha/year)	Comparison rate (%)
1	Chan Thinh commune, Van Chan district	42.59	33.26	121.91
2	Viet Thanh commune, Tran Yen district	41.23	32.21	121.88
	Average	41.91	32.74	121.89

3.2. The impact of silkworm rearing technology to cocoon yield and income earned

3.2.1. Young age silkworm reared by Cooperatives

Young age silkworm of new double cross silkworm variety coded VH2020 was reared by 3 Cooperatives namely the Green Environment Cooperative of Chan Thinh commune (Tu famer), Son Thinh Sericulture Service Cooperative (Mong farmer) and Viet Thanh Sericulture Cooperative to provide fourth instar silkworms to famersin the demonstrations.

Table 5. Quantity of young agesilkworm reared by cooperative in 2020 - 2022 period

No.	Locations	Year	Number of harvests	Quantity of silkworm egg (egg card)
1	Van Chan district (Son Thinh commune, Chan Thinh commune)	2020	10	1,809
		2021	12	3,777
		2022	12	3,966
Sub- total				9,552
2	Tran Yen district (Viet Thanh commune)	2020	-	-
		2021	6	397
		2022	8	1,613
Sub- total				2,010
Total				11,562

Notes: (-) The project was started in Tran Yen district in 2021.

During 2020 – 2022, 11,562 silkworm egg cards were delivered to farmers at Van Chan and Tran Yen district (Table 5). Silkworm egg had good quality, hatching ratio of silkworm egg was reported more than 90% and free disease young age silkworm of good quality was also obtained.

3.2.2. Late age silkworm rearing

In the scale of the project, farmers who reared silkworm come from 3 communes (Chan Thinh, Son Thinh and Viet Thanh). They were trained by skilled experts and provided with sufficient quantity of disinfectants, preventive drugs and young age silkworm as well. In addition, farmers were also instructed on the detailed techniques of spreading silkworms on the floor by rows and how to feed and take care of silkworms at different stages including molting one.

In general, the cocoon yield in 2022 was in significantly higher compared to 2021 in Chan Thinh demonstration and no high fluctuation from harvest to harvest was also recorded (Table 6).

Table 6. Yield of silkworm cocoon in different demonstrations in 2020 - 2022 period

No.	Location	Year	Quantity (egg card)	Cocoon Yield (kg/card)	Cocoon yield (ton/ha/year)
1	Chan Thinh commune, Van Chan district	2020	1,809	9.84	1.18
		2021	2,514	10.12	1.12
		2022	2,526	10.83	1.30
2	Son Thinh commune, Van Chan district	2020	-	-	
		2021	986	9.05	1.09
		2022	1,416	10.02	1.20
3	Viet Thanh commune, Tran Yen district	2021	397	10.50	1.26
		2022	1,613	11.10	1.33

It is also mentioned that the skill of farmers participated in the project in terms of silkworm rearing technology significantly improved, resulting in an increase in the cocoon yield year by year.

Data summarized in table 7 showed that cocoon yield gradually increased. In addition, the cocoon price increased considerably during 2021 - 2022, whichhelped increase the income of producers. To illustrate, in Son Thinh commune, theincome earned from a silkworm egg card in 2022 increased by 19.52% compared to 2021.

Table 7. Average in come earned from a silkworm egg card in 2020 - 2022 period

No.	Locations	Year	Cocoon yield (kg/card)	Cocoon price/kg (VND)	Average in come/silkworm egg card (VND)	Production value increase (VND)
1	Chan Thinh commune, Van Chan district	2020	9.84	92,000	905,280	-
		2021	10.23	120,000	1,227,600	322,320
		2022	10.83	129,000	1,297500	169,900
		Comparison 2021 to 2022	5.89	7.50	13.84	
2	Son Thinh commune, Van Chan district	2020	-	-	0	
		2021	9.47	108,000	1,022,760	-
		2022	9.86	122,000	1,212,420	199,680
		Comparison 2021 to 2022	5.81	12.96	19.52	

In Chan Thinh commune:

- As a matter of fact, the cocoon yield of silkworm variety LQ2 (Chinese variety), reared by farmers who did not participate in the project (outside of the demonstrations) was much lower than domestic ones reared by project participants (9.08 compared to 10.23 kg/egg card, 13% fluctuation). Therefore, the income for 1 egg card in the demonstrations was much higher than outside fields (356,200 VND increased; table 8)

Table 8. Average in come earned from a silkworm egg card in 2021 at Chan Thinh commune

No.	Types	Cocoon yield (kg/eggcard)	Cocoon yield increased (%)	Average in come/ egg card (VND)	Average in come/egg card increased (VND)
1	Demonstration	10.23	113	1,27,900	356,200
3	Normal fields	9.08		871,680	-

3.3. Impact of improved mountage types to the quality of cocoon and raw silk

With the assistance from skilled technicians from research institutions, newly improved mountages were done by the participating farmers who have been instructed with the techniques concerned, such as the way to place ripen silkworms crawl on the mountages, how to remove cocoon, etc. Data summarized in table 9 proved that the types of mountages significantly affected the quality of cocoon.

Table 9. The effect of the mountage to cocoon quality in Chan Thinh commune

No.	Contents	Bamboo mountage	Wooden square mountage
1	Cocoon whole weight (g)	1.46	1.61
2	Cocoon shell ratio (%)	18.19	19.37
3	Double cocoon ratio (%)	6.12	1.77
4	Silk length (m)	774	871
5	Reelibility (%)	76.72	84.10
6	Silkwaste percentage (%)	1.80	1.62
7	Rendital (kg)	7.86	7.04
8	Grate of cocoon quality	5G/10G	6G/10G

It is obvious that the types of mountage affected not only the quality of cocoon but also the yield of silk and cocoon, even when silkworms had already stopped eating mulberries in the cocooning process. For the mountages made of hygroscopic materials and suitable space for cocooning, silkworms produced cocoons easily, spent less quantity of original silk to shape the cocoon shell and higher yield of cocoon with better silk quality was also reported. Among the types of mountages tested, wooden square mountage was considered to have many advantages. The cocoons obtained in this type of mountage were quite uniform and clean due to the limitation of yellow stains secreted by silkworms. The cocoon yield was 10.24% higher than the control while the cocoon harvesting time reduced to 67.19%. Moreover, the rate of good cocoon increased by 7.19%, very few double cocoon and waste cocoon was reported. All the criteria related to cocoons quality obtained in wooden square mountages were better than the others, indicated by the length of cocoon filament (12.62% longer), the reelability (11.06% higher), cocoon quality ranked at 6G level, where as reeling silk size of 20 – 22 D was already recorded from cocoons harvested in wooden square mountage which was evaluated at grade 2A, better than bamboo mountage (3A grade) [9].

Since press tool can be used for taking cocoons out from wooden square mountage, the time to harvest would be shorter than W bamboo mountage and withdraw bamboo one that have to be done by hand.

Besides, the cocoon yields in wooden square mountage were higher than withdraw bamboo mountage (10.24%).

Reelability is an indicator that depends on the environmental conditions in which temperature and relative humidity during the larvea spinning play an important role.

Table 10. Effect of mountage type to silk quality (20 - 22D raw silk)

No.		W bamboo mountage		Wooden square mountage		Withdraw bamboo mountage (control)	
		Reality	Grade	Reality	Grade	Reality	Grade
1	Average Conditioned Size (D)	20.21	Good	20.37	Good	19.78	Good
2	Size Deviation (den)	2.2	A	1.81	2A	1.96	A
3	Maximum Sizing Deviation (den)	4.71	3A	3.98	4A	4.26	4A
4	Cleanness	88	2A	89.50	2A	87.67	A
5	Neatness	97.60	5A	98.90	6A	96.83	4A
6	Appearance Inspection	No white colour, Soft		White colour, Soft		No white colour	
7	GRADE	A		2A		A	

Using wooden square mountage, the cocoon quality was highly appreciated, asindicated by higher ratios of cocoon shell, reelability, rendital compared to bamboo mountage.

In short, it can be said that mountage types had a significant impact on the yield and quality of cocoon produced by silkworms. As a result, wooden square mountages have been popularlyutilized in most silkworm farms in Van Chan and Tran Yen districts of Yen Bai province (Table 11).

Table 11. Percentage of farmer using wooden square mountage to rearing silkworm in Yen Bai province

No.	Locations	2020	2021	2022
1	Chan Thinh commune, Van Chan district	20	80	100
2	Son Thinh commune, Van Chan district	20	50	100
3	Viet Thanh commune, Tran Yen district	80	100	100

4. CONCLUSION

The application of technical advances in the form of over-all demonstrations had apositive effect on sericulture production in Yen Bai province, indicated by improving the heath of mulberry (21.89% higher leaf yield compared to the control), the yield of cocoon (13% higher than the control); the shortening of harvest duration (67.9% shorter than usual) and the quality of cocoon (7.19% higher in goodcocoon ratio than the control).

Utilization of wooden square mountage in rearing silkworm remarkably improved remarkably the quality of cocoon released and this device has been used by nearly 100% sericulture farmers up to now.

The demonstrations of over-all technical advances increased significantly the income of sericulture farmers, thus improving their living standards.

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STUDY ON NEW BIOPESTICIDES VAAS-AT1 AND VAAS - AT2 TO CONTROL THE PEPPER ROOT ROT AND ROOT - KNOT NEMATODE DISEASE AND COFFEE YELLOW LEAF AND ROOT ROT DISEASE IN VIETNAM

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ABSTRACT

This study aimed at evaluating the efficacy of biopesticides containing nematophagous fungi and antagonistic microorganisms, named VAAS-AT1 and VAAS-AT2, for controllingplant-parasitic nematodes and pathogenic fungi causing root rot and root-knot nematode disease complex in black pepper plants and yellow leaf and root rot diseases in coffee plants in Vietnam. A series of experiments were conducted in greenhouses, small-scale experiments, and field demonstrations. The results showed that both biopesticides effectively reduced the population of plant-parasitic nematodes and pathogenic fungi in the soils growing with black pepper and coffee plants. In greenhouse conditions, VAAS-AT1 demonstrated an efficacy of 83.3% against *Phytophthoracapsici,* 79.5% against *Fusarium solani*, 60.6% against *Rhizoctonia solani,* and 77.8% against plant-parasitic nematodes after 3 months of treatment. In small-scale field trials, VAAS-AT1 achieved efficacies ranging from 81.3 to 83.5% against *Phytophthora* spp., 82.3 to 83.7% against *Fusarium* spp., and 80.3 to 81.9% against plant-parasitic nematodes after 9 months of treatment. In field demonstrations, the efficacy of VAAS-AT1 reached up to 79.3% in Dak Lak province and 77.6% in Gia Lai province against the pepper root rot disease after 9 months of treatment. VAAS-AT2 exhibited its efficacy in greenhouse conditions by reducing the population of plant-parasitic nematodes by 69.82% and *F. oxysporum* by 76.44% after 3 months of treatments. In small-scale field experiments, VAAS-AT2 achieved efficacies of 76.16 to 78.12% against plant-parasitic nematode and 78.04 to 79.26% against *Fusarium* spp. after 9 months of treatment. In the field demonstrations, the efficacy of VAAS-AT2 against yellow leaf disease in coffee plants reached 72.73 to 79.3% after 9 months of treatment in Dak Lak and Gia Lai provinces, respectively. Furthermore, the economic benefits of using VAAS-AT1 and VAAS-AT2 were substantial, with income increases of 17.89 to 25.80% for farmers and a benefit over control ranging from 5.260 to 22.500 million VND/ha. These findings demonstrate the promise of VAAS-AT1 and VAAS-AT2 as effective biopesticides for enhancing crop health and income for farmers in Vietnam.

Keywords: *Bio-pesticide, black pepper, coffee, root rot and root-knot nematode, VAAS-AT1, VAAS-AT2, yellow leaf and root rot.*

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1. INTRODUCTION

Black pepper and coffee industries play a vital role in Vietnam's economy, with the export values reaching USD 1.422 billion and 2.862 billion in 2019 [1]. However, the production of black pepper and coffee in Vietnam faces numerous challenges, including price fluctuation in international markets, the impact of climate change, and, notably, the emergence of destructive soil-borne diseases.

One of the biggest obstacles and costly burdens in agricultural crop production is plant-parasitic nematodes, which result in substantial economic losses estimated at USD 80 to 118 billion annually [2]. As reported by Yadav in 2017 [3], these

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nematodes cause an average yield loss of over 10% globally; forcertain crops, this loss exceeds 20%. In tropical and sub-tropical climates, the impact of plant-parasitic nematode is particularly pronounced, causing a crop production loss of about 14.6%, in contrast to 8.8% in developed countries. Additionally, plant-parasitic nematode spose a significant challenge to coffee growers, as estimated by Youssef in 2013 [4], with annual crop losses amounting to approximately USD 157 billion.The damage inflicted by nematodes often paves the way for secondary infection by soil-borne pathogens, including *Fusarium* spp., *Phytophthora* spp., and *Rhizoctonia* spp., among others. This leads to the complete deterioration of the root system, resulting in root rot and, ultimately, plant death [5, 6, 7].

Various species of fungi and bacteria have been identified as biocontrol agents for combating plant-parasitic nematodes and pathogenic fungi, as documented in previous studies [8, 9, 10, 11, 12, 13]. Biopesticides represent a sustainable approach within integrated pest management, with minimal environmental impact and no adverse effects on human health or animals. However, despite these benefits, the commercialization of biopesticides still needs to be improved. In 2017, the proportion of biopesticides used in agriculture compared to conventional chemical pesticides was estimated at around 5% [14]. This indicates a significant opportunity for expanding and adopting biopesticide solutions in agricultural practices.

Several biopesticides have been registered and authorized in Vietnam as plant protection agents. According to Circular No. 19/2020/TT-BNNPTNT, there are 16 active ingredients with 20 trade names derived from various bio-agents, including *Trichoderma, Streptomyces, Bacillus, Metarhizium, Paecilomyces*, and *Chaetomium.* These biopesticides have been recommended for controlling plant-parasitic nematodes and pathogenic fungi in cultivatingblack pepper and coffee plants [15]. Most currently available commercial biopesticidesare produced from one or more strains of antagonistic or nematophagous microorganisms of the same species, with or without the addition ofbiologically active ingredients. However, they do not contain a wide variety of biological agents. Consequently, the effectiveness of these biopesticides in controlling plant-parasitic nematodes and pathogenic fungi remains relatively low,mainly due to the influence of numerous biotic and abiotic factors.

Within the framework of national research projects aimed at developing new biopesticide for controlling plant-parasitic nematodes and pathogenic fungi that damage coffee and black pepper in Vietnam, the Vietnam Academy of Agricultural Sciences, in collaboration with its member institutes (Soils and Fertilizers Research Institute - SFRI, Plant Protection Research Institute - PPRI, Institute for Agricultural Environment - IAE, Agricultural Genetics Institute – AGI and Western Highlands Agriculture and Forestry Science Institute - WASI), has undertaken a comprehensive effort. This initiative involved the selection, identification, and evaluation of the efficacy of a diverse group of antagonistic microorganisms and nematophagous fungi, including *Purpureocillium lilacinum* Pae, *Arthrobotrys oligospora* NVC7.4, *Bacillus velezensis* P9.1, *Streptomyces enissocaesilis* 2P, *Chaetomium cochliodes* TQHT01, *Trichoderma asperellum* Trivass. The research has culminated in developing a technology for producing novel biopesticides named VAAS-AT1 for black pepper plants and VAAS-AT2 for coffee plants. These biopesticides harbor a beneficial microorganism density of 10^8 CFU/g [16].

The objective of this study was to evaluate the effectiveness ofthe newly developed biopesticides, VAAS-AT1 and VAAS-AT2, in controllingroot rot and root-knot nematodes disease complex in black pepper plants, as well as the yellow leaf and root rot disease of coffee plants in Vietnam.

2. MATERIALS AND METHODS

VAAS-AT1 was applied toblack pepper plants, while VAAS-AT2 was used for coffee plants, and both were produced using selected nematophagous fungi and antagonistic microorganisms with a beneficial microorganism density of 10^8 CFU/g. *Pratylenchus coffeae, Meloidogyne incognita, Phytophthora capsisi, F. solani, Fusariumoxysporum,* and *R. solani* were provided by the Plant Protection Research Institute. Coffee plants of the Tr4 variety and Vinh Linh black pepper variety were sourced from the Western Highlands Agriculture and Forestry Science Institute.

Greenhouse experiments wereconducted using sterilized soil, which wassubsequently infected with pathogenic fungi including *P. capsisi, F. solani, F.*

oxysporum, R. solani, and nematodes such as *P. coffeae, M. incognita.* VAAS-AT1 or VAAS-AT2 was inoculated when planting black pepper and coffee, while the control treatment did not receive this inoculation.

The soil contained a density of 10^4 CFU/g soil of pathogenic fungi, antagonistic microorganisms, and nematophagous fungi, along with 250 total nematodes at the adult stageper gram of soil. The density of pathogenic fungi and nematodes in the soil was monitored one and three months after the treatments. The density of plant-parasitic nematodes in the black pepper and coffee growing soils was determined using the method described by Ha and Ton (2011) [17], while the density of pathogenic fungi in the soils was calculated following the approach outlined by Burgess *et al.* (2009) [18]. Additionally, the density of *Phytophthora* spp.. was evaluated using the method described by Erwin and Riberrio (1996) [19]. The reduction percentages of plant-parasitic nematodes and pathogenic fungi were calculated using Henderson Tilton's formula:

$$\% \text{ population reduction} = (1\text{-})\frac{T_a \times C_b}{C_a \times T_b} \times 100$$

Where: T_a=number of plant-parasitic nematodes or pathogenic fungi after treatment; T_b=number of plant-parasitic nematodes or pathogenicfungi before treatment; C_a=number of plant-parasitic nematodes or pathogenicfungi in control plots after treatment; C_b=number of plant-parasitic nematodes or pathogenicfungi in control plots before treatment.

The small-scale field experiments were carried out according to the Ministry of Agriculture and Rural Development regulations, following an integrated crop management system at both the young and mature stages of the coffee and black pepper plants. The treatments included: 1) Negative control, reflectingtypical farmer practices. 2) Application of VAAS-AT1 or VAAS-AT2 at a dose of 30 g per plant. The experiments were designed with 30 plants per treatment and organized into 3 replications. The density of plant-parasitic nematodes and pathogenic fungi in the soil was monitored after 3, 6, and 9 months of treatment. The reduction percentages were calculated using Henderson-Tilton's formula.

Two field demonstrations for each crop were established in Gia Lai and Dak Lak provinces, with two treatmentswithout replication, including 1) Farmer practices and 2) Application of VAAS-AT1 or VAAS-AT2 at a dose of 30g per plant. The total area of the field demonstration was 1 ha per location. The rate of damaging plants (efficacy) was monitored 1, 3, 6, and 9 months after treatment following the guidelines of TCVN 12561: 2018: Pesticides - Bio-efficacy field trials [20]. The efficacy (E) was calculated using the formula:

$$E(\%) = (1 - \frac{Ta}{Ca}) \times 100$$

Where: E(%) = efficacy, Ca = disease severityin the farmer practices. Ta = disease severityin the treatment.

The disease severity was evaluated using a disease rating scale from 0 to 4 as follows: Scale 0: no symptom; scale 1(N_1): less than 25% of leaves turned yellow; Scale 2 (N_2): between 25 and less than 50% of leaves turned yellow; Scale 3 (N_3): Between 50 and 75% of leaves turned yellow; Scale 4 (N_4): more than 75% of leaves turned yellow. The disease severity in each treatment was calculated using the formula:

$$\text{Disease severity (\%)} = \frac{(N_1 \times 1) + (N_2 \times 2) + (N_3 \times 3) + (N_4 \times 4)}{4 \times \text{number of surveyed plants}} \times 100$$

Where: N_1, N_2, N_3, and N_4: number of damaging plants of each scale.

All optimization studies were carried out, and the data was analyzed using single-factor analysis of variance (ANOVA). The data were all summarized as mean ± SD (standard deviation). Mean separation was performed using the Duncan test at P=0.05 whenever a significant ANOVA (P< 0.05) result occurred or was processed according to the IRRISTAT statistical and data processing program.

3. RESULTS

3.1. Efficacy of VAAS-AT1 in controllingplant-parasitic nematodes and pathogenic fungion black pepper

The results of the greenhouse experiments evaluating the efficacy of VAAS-AT1 in controlling plant-parasitic nematodes and pathogenic fungi on black pepper are summarized in table 1. The results indicated that VAAS-AT1effectively controlled both plant-parasitic nematodes and pathogenic fungi. After 3 months of treatment, VAAS-AT1 achieved remarkable efficacy with 83.3% against *P. capsici,* 79.5% against *F. solani,* 60.6% against *R. solani,* and 77.8% against plant-parasitic nematodes.

Table 1. Efficacy of VAAS-AT1 in controlling plant-parasitic nematodes and pathogenic fungi in soil cultivating black pepper *(Thegreenhouse experiment, 2019)*

Treatments	The population of pathogens in the soil after treatment of		Efficacy (%) after 3 months of treatment
	01 month	03 months	
	Phytophthora spp. *		
P. capsici	60.0c	63.3c	-
P. capsici + M. incognita	63.3c	70.0d	-
P. capsici + M. incognita + VAAS-AT1	15.0a	11.7ab	83.3
	Fusarium spp.density (10^3CFU/g soil)		
F. solani	8.0b	12.3b	-
F. solani + M. incognita	8.3b	13.0b	-
F. solani + M. incognita + VAAS-AT1	3.0a	2.7a	79.5
	Rhizoctonia spp. density (10^3CFU/g soil)		
R. solani	5.3c	7.7b	-
R. solani + M. incognita	6.0d	11.0c	-
R. solani + M. incognita + VAAS-AT1	2.7ab	4.3a	60.6
	Nematode density (numbers of total nematodes/100 g soil)		
P. capsici + M. incognita	467.7c	717.0b	-
P. capsici + M. incognita + VAAS-AT1	177.3a	159.3a	77.8

*Statistical analysis indicated that averages not followed by the same lowercase letter within columns are significantly different at a significance level of P<0.05, as determined by Duncan's multiple range test. *The density of Phytophthora spp. was evaluated based on the number of rose petals losing their color.*

The field trials on black pepper plants in WASI indicated that VAAS-AT1 significantly reduced the density of plant-parasitic nematodes and pathogenic fungi. Notably, at the young stage, its efficacy was remarkable, achieving 81.3% against *Phytophthora* spp., 82.3% against *Fusarium* spp., 81.9% against plant-parasitic nematodes. Similarly, at the maturity stage, after 9 months of treatment, VAAS-AT1 continued to exhibit high efficacy of 83.5% against *Phytophthora* spp., 83.7% against *Fusarium* spp., and 80.3% againstplant - parasitic nematodes, all incomparison to the control (Table 2).

Table 2. Efficacy of VAAS-AT1 in controlling plant-parasitic nematodes and pathogenic fungi in soil-growing black pepper plants *(The small field trials, 2019)*

Pepper plant at the young stage				
Treatments	The population of pathogens in the soil after treatment of			Efficacy (%) after 9 months of treatment
	03 months	06 months	09 months	
	Phytophthora spp. *			
VAAS-AT1	21.0	13.0	15.0	81.3

Control (non-treatment)	71.0	74.0	80.0	
CV(%)	16.8	10.6	11.7	
$LSD_{0.05}$	7.8	4.0	4.1	
	Fusarium spp. density (10^3CFU/g soil)			
VAAS-AT1	4.4	4.2	4.4	82.3
Control (non-treatment)	17.2	23.2	24.8	-
CV(%)	28.2	21.3	21.0	
$LSD_{0.05}$	3.0	2.6	2.7	
	Nematode density (nematodes/100 g soil)			
VAAS-AT1	453.7	176.7	283.0	81.9
Control (non-treatment)	1291.3	1022.7	1564.0	-
CV(%)	13.3	14.1	8.0	
$LSD_{0.05}$	180.5	110.6	95.9	
Pepper plants at the maturity stage				
Treatments	The population of pathogens in the soil after treatment of			Efficacy (%) after 9 months of treatment
	03 months	06 months	09 months	
	Phytophthora spp. *			
VAAS-AT1	6.0	2.8	2.8	83.5
Control (non-treatment)	18.4	15.2	17.0	
CV(%)	9.7	7.5	6.0	
$LSD_{0.05}$	8.6	4.9	4.3	
	Fusarium spp. density (10^3CFU/g soil)			
VAAS-AT1	9.2	4.2	6.0	83.7
Control (non-treatment)	31.0	22.6	36.8	-
CV(%)	28.0	20.4	19.6	
$LSD_{0.05}$	8.2	4.0	6.1	
	Nematode density (nematodes/100 g soil)			
VAAS-AT1	571.0	234.3	262.7	80.3
Control (non-treatment)	1265.3	1099.3	1335.7	-
CV(%)	7.7	14.2	7.8	
$LSD_{0.05}$	159.5	217.0	141.5	

**The population of Phytophthora spp. was evaluated as the number of rose petals losing their color.*

In the field demonstration of black pepper plantations in Gia Lai and Dak Lak provinces, the effectiveness of VAAS-AT1 was markedly superior to the farmers' conventional practices. The application of VAAS-AT1 resulted in a notable reduction in disease incidence and severity. Specifically, in Dak Lak province, disease incidence decreased from 1.31 to 0.62%,while disease severity dropped from 0.56 to 0.17% after 9 months of treatment. In Gia Lai province,the impact was even more significant, as disease incidence decreased from 17.60 to 6.40%, and disease severity reduced from 6.40 to 1.6% over the same period. The efficacy of VAAS-AT1 reached 79.3% in Dak Lak province and 77.6% in Gia Lai province after 9 months of treatment (Table 3).

Table 3. The efficacy of VAAS-AT1 on the plant parasitic nematodes of black pepper plants (*The field demonstrations, 2019 – 2020*)

Time of survey	Farmers' practices		Demonstration (VAAS-AT1 treated)		
	Disease incidence	Disease severity	Disease incidence	Disease severity	Efficacy (%)
Dak Lak province					
Day before treatment (Jun., 2019)	0.15	0.04	0.23	0.06	
1 month after treatment (Jul., 2019)	0.23	0.08	0.23	0.06	50.0
3 months after treatment (Sep., 2019)	0.77	0.35	0.38	0.12	77.8
6 months after treatment (Dec., 2019)	1.08	0.50	0.46	0.13	82.1
9 months after treatment (Mar., 2020)	1.31	0.56	0.62	0.17	79.3
Gia Lai province					
Day before treatment (Jun., 2019)	10.40	2.72	11.20	3.04	-
1 month after treatment (Jul., 2019)	15.20	4.64	11.20	3.04	41.4
3 months after treatment (Sep., 2019)	12.00	4.16	4.80	1.12	75.9
6 months after treatment (Dec., 2019)	13.60	4.96	4.00	0.96	82.7
9 months after treatment (Mar., 2020)	17.60	6.40	6.40	1.60	77.6

In Gia Lai province, the pepper corns yield reached 4 tons per hectare, while in Dak Lak province, it was even more substantial at 5.6 tons per hectare. As a result of implementing VAAS-AT1, farmers got a significant economic benefit, with income increases ranging from 21.900 million to 22.500 million VND per hectare, which equated to a notable income rise of 17.89 to 25.8% when compared to traditional farmer practices (Table 4).

Table 4. Economic efficiency of VAAS-AT1application in black pepper production *(Field demonstrations, 2019 - 2020)*

Items	Gia Lai province		Dak Lak province	
	Farmers' practices	Demonstration	Farmers' practices	Demonstration
Total input (million VND)	55.200	52.300	68.000	64.500
Total outcome (million VND)	140.000	160.000	193.800	212.800
Benefit (million VND)	84.800	107.700	125.800	148.300
Benefit over control (million VND)	-	21.900	-	22.500
Benefit over control (%)	-	25.80		17.89

Notice: Peppercornsprice: 38,000 VND per kg

3.2. Efficacy of VAAS-AT2 on controlling plant-parasitic nematodes and pathogenic fungiin coffee plants

The greenhouse experiments aimed at evaluating the effect of VAAS-AT2 on the population of plant-parasitic nematodes and pathogenic fungi in soil-growing coffee plants are summarized in table 5. The results indicated that VAAS-AT2is highly effective in reducing the population of plant-parasitic nematodes and pathogenic fungi in the soil. Specifically, after just 3 months of treatment, VAAS-AT2 led to a remarkable 69.82% reduction in the population of plant-parasitic nematodes in the soil. A similar trend was observed in the reduction of *F. oxysporum* population, which reached an impressive 76.44% reduction following the treatments.

Table 5. The effect of the VAAS-AT2 on the population of pathogenic fungi and nematodes in the coffee plants-growing soil ***(Greenhouse experiments, 2019)***

Treatments	Nematode density		
	Number of nematodes per 100 gram of soil after treatment		Population reduction compared to control after 3 months (%)
	01 month	03 months	
F. oxysporum (control)	-	-	-
P. coffeae (control)	285.67	318.82	-
F. oxysporum + *P. coffeae* +VAAS-AT2	135.27	127.12	69.82
CV(%)	2.30	2.10	-
$LSD_{0.05}$	7.51	6.75	-
Treatments	*F. oxysporum* density		
	10^3 CFU per gram of soil after treatment		Population reduction compared to control after 3 months (%)
	01 month	03 months	
F. oxysporum (control)	75.23	91.56	-
P. coffeae (control)	-	-	-
F. oxysporum + *P. coffeae* +VAAS-AT2	35.16	31.17	76.44
CV(%)	4.60	2.00	-
$LSD_{0.05}$	3.83	1.60	-

The small-scale field study examining the impact of VAAS-AT2 showed that the plant-parasitic nematode population was significantly reduced. At the early stage of coffee plantations, there was a substantial reduction of 77.16%, and at the maturity stage, this reduction increased to 78.12% after 9 months of treatment. A similar positive trend was observed in the reduction of *Fusarium* spp. population in coffee plantations, with a reduction of 78.04% at the young stage and an even more significant reduction of 79.26% at the maturity stage (Table 6).

Table 6. Effect of VAAS-AT2 on the population of plant-parasitic nematode and pathogenic fungi in the coffee-growing soil ***(Small-scale field experiments, 2019)***

Treatments	Coffee plant at the young stage after treatment			
	03 months	06 months	09 months	Population reduction after 9 months (%)
	Nematode density (Number of nematodes/100 g of soil)			
Control	385.4	410.5	430.8	-
VAAS-AT2	108.5	100.6	98.4	77.16
CV(%)	1.8	3.1	3.3	
$LSD_{0.05}$	7.40	1.07	1.32	
	Fusarium spp. density (10^3CFU/g soil)			
Control	85.9	92.37	95.73	-
VAAS-AT2	25.47	21.36	21.02	78.04
CV(%)	1.3	2.6	1.6	
$LSD_{0.05}$	1.16	2.11	1.31	

	Coffee plant at the maturity stage after treatment			
Treatments	03 months	06 months	09 months	Population reduction after 9 months (%)
	Number nematodes/100 g of soil (nematodes/g)			
Control	382.7	415.60	435.80	-
VAAS-AT2	118.2	106.37	95.36	78.12
CV(%)	0.9	1.4	2.8	
$LSD_{0.05}$	3.27	5.27	4.55	
	Fusarium spp. density (10^3CFU/g soil)			
Control	46.27	52.74	61.52	-
VAAS-AT2	15.37	13.27	12.76	79.26
CV(%)	3.7	3.3	2.2	
$LSD_{0.05}$	1.94	1.67	1.17	

In the field demonstrations conducted on coffee plantations in Dak Lak and Gia Lai provinces, the efficacy of VAAS-AT2 in controlling the yellow leaf disease of coffee plants reached an impressive 72.73 to 79.3% after 9 months of treatment with VAAS-AT2 (Table 7).

Farmers who applied VAAS-AT2 enjoyed a substantial economic benefit over the control, with an income increase from 5.260 to 9.400 million VND per hectare, which equaled a remarkable income boost of 21.75 to 27.62% compared to the control practices (Table 8).

Table 7. Efficacy of VAAS-AT2in controlling yellow leaf disease in coffee plants (*Field demonstrations, 2019-2020*)

Time of survey	Disease incidence		Efficacy (%)
	Farmers' practices	Demonstration	
Dak Lak province			
Day before treatment (Jun., 2019)	0.15	0.23	-
1 month after treatment (Jul., 2019)	0.23	0.23	50.0
3 months after treatment (Sep., 2019)	0.77	0.38	77.8
6 months after treatment (Dec., 2019)	1.08	0.46	82.1
9 months after treatment (Mar., 2020)	1.31	0.62	79.3
Gia Lai province			
Day before treatment (Jun., 2019)	14.40	12.00	-
1 month after treatment (Jul., 2019)	14.67	6.67	53.3
3 months after treatment (Sep., 2019)	16.00	6.00	60.6
6 months after treatment (Dec., 2019)	16.67	4.00	76.4
9 months after treatment (Mar., 2020)	15.33	4.00	72.73

Table 8. Economic efficiency of VAAS-AT2 in coffee production *(Field demonstrations, 2019-2020)*

Items	Gia Lai province		Dak Lak province	
	Control	Demonstration	Control	Demonstration
Total input (million VND)	55.800	53.000	44.000	44.500
Total outcome (million VND)	99.000	105.600	63.040	68.800
Benefit (million VND)	43.200	52.600	19.040	24.300
Benefit over control (million VND)	-	9.400	-	5.260
Benefit over control (%)	-	21.75		27.62

Notice: Coffee bean price: 33,000 VND/kg;

4. DISCUSSION AND CONCLUSION

Beneficial microorganisms have emerged as valuable bio-agents for controlling plant diseases, offering a reliable and environmentally friendly alternative to chemical pesticides in agricultural production. Microbial biopesticide represents an economically viable and eco-friendly strategy for managinga range of plant diseases and insect pests affecting various crops [14]. Traditionally, microbial biopesticide typically consisted of a single strain of microorganisms. However, the effectiveness and persistence of a single microbial inoculant are profoundly influenced by factors such as soil microbiota and environmental conditions, resultingin inconsistency and inefficacy [21, 22, 23]. The development of microbial biopesticides has shifted toward microbial consortia as a promising solution to address these challenges. Combining compatible consortia of multifunctional microorganisms holds the potential to effectively control soil-borne pathogens through both direct and plant-mediated disease suppression. Synthetic microbial consortia offer a promising strategy to enhance the functionality and versatility of microbial biological control [22]. These microbial consortia, composed of multiple functional microorganisms, have found wide application in agricultural production. They have successfully controlled soil-borne diseases in valuable crops by competing for nutrient resources and niches with pathogenic microorganisms. Additionally, they producevarious antimicrobial compounds, facilitate the development of systemic resistance, and regulate soil microbial communities. Previous studies have reported enhanced stability and efficacy when using microbial consortia compared to individual microorganisms [24, 25, 26].

The efficacy of microbial consortia, which include combinations of different beneficial microorganisms, has been demonstrated in various studies. For example, a consortium of *T. asperellum* GDFS1009 and *B. amyloliquefaciens* ACCC1111060 strains showed higher efficacy against *Botrytis cinerea* than individualstrains [27]. Similarly, the combination of *T. virens* GI006 and *B. velezensis* Bs006 was more efficient in combating the *Fusarium* wilt of cape gooseberry when compared to usingthe individual strains [28].

The utilization of microbial consortia represents a significant advancement in biopesticides. These consortia, composed of multiple beneficial microorganisms, offer a more consistent and practical approach to disease management, providing a sustainable and eco-friendly solution for modern agricultural challenges. The findings presented in this study, particularly the efficacy of VAAS-AT1 and VAAS-AT2 in controlling plant-parasitic nematodes and pathogenic fungi in black pepper and coffee plants, demonstrate the potential of these microbial biopesticides to benefit crop production in Vietnam. This research lays the foundation for further exploration and development of microbial consortia for disease control and sustainable agriculture.

In the present study,we evaluated the new biopesticides named VAAS-AT1, and VAAS-AT2 for controlling plant-parasitic nematodes and pathogenic fungi on black pepper and coffee plants in Vietnam. The results indicated that VAAS-AT1 and VAAS-AT2 significantly reduce the population of plant-parasitic nematodes and pathogenic fungi in soils-growing black pepper and coffee plants. Forblack pepper plants, VAAS-AT1 exhibited a remarkable efficacy of 83.3% against *P. capsici*, 79.5% against *F. solani*, 60.6% against *R. solani*, and 77.8% against plant-parasitic nematodes after 3 months of treatment in the greenhouse condition. Its efficacy was 81.3 to 83.5% against *Phytophthora* spp., 82.3 to 83.7% against *Fusarium* spp., and 80.3 - 81.9% against plant-parasitic nematodes after 9 months of treatment in small-scale field experiments. In the field demonstration, the efficacy of VAAS-AT1 reached 79.3% in Dak Lak province and 82.7% in Gia Lai province against root-knot disease in black pepper plants after 9 months of treatment. VAAS-AT2 reduced the population of plant-parasitic nematodes by 69.82% and the *F. oxysporum* population by 76.44% after 3 months of treatments in greenhouse conditions. In small-scale field experiments, its efficacy remained high at 77.16 to 78.12% in the nematode population and 78.04 to 79.26% in *Fusarium* spp. population after 9 months of treatment. In the field demonstrations on coffee plantations in Dak Lak and Gia Lai provinces, VAAS-AT2 achieved an efficacy of 72.73 to 82.1% in controlling yellow leaf disease after 9 months of treatment. The economic benefits of using VAAS-AT1 and VAAS-AT2 were substantial, with income increases ranging from 21.900 to 22.500 million VND per hectare, equivalent to an income increase of 17.89-25.8% and 5.260 to 9.400 million VND per hectare, equivalent to an income growth of 21.75 to 27.62%, respectively. These research results strongly indicate that VAAS-AT1 and VAAS-AT2 can serve as effective new biopesticides for controlling the complex black pepper root rot, root-knot nematode diseases, and coffee yellow leaf and root rot disease. These findings promise to enhance crop production in Vietnam and present a significant advancement in sustainable agriculture practices.

Nevertheless, developing VAAS-AT1 and VAAS-AT2 for controlling soil-borne plant-parasitic nematodes and pathogenic fungi on black pepper and coffee plants requires additional research. This research should encompass various environmental properties, and climatic conditionsto understand the dynamic interrelationship of nematophagous fungi and antagonistic microorganisms in different ecosystems. Conducting official field trials, overseenby designated organizations, is essentialtoenhance the quality of VAAS-AT1 and VAAS-AT2by refining their production technology. These efforts will further contribute to the practical and sustainable managing diseases in black pepper and coffee plants.

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IMPLEMENTATION RESULTS OF THE NATIONAL RICE PRODUCT DEVELOPMENT PROJECT UP TO THE YEAR 2020 AND ORIENTATION OF PRODUCT RESEARCH AND DEVELOPMENT TO 2030

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ABSTRACT

Rice (*Oryza sativa*) has been regarded as one of the most important food crops that plays an important role in the socio-economic development of Vietnam. Ensuring food security and promoting sustainable rice production and export are two crucial tasks. This report provides an implementation result of the National Rice Product development project up to the year 2020 and anorientation of product research and development to 2030. Particularly, a total of 11 and five new high-grade rice varieties and promising lines have been constructed, respectively. Among them, eight rice varieties, including ĐH12, OM429, BĐR27, GL516, OM375, OM20, OM08, and HD11, were transferred to enterprises and developed in various ecological regions. Furthermore, advanced cultivation practices have been constructed for the Northern provinces, the Red River Delta, the North and South Central regions, and the Mekong River Delta. Certified seeds, registered seeds and basis seeds, together with advanced production models, were developed in many provinces to ensure the production of high-grade rice varieties in Vietnam. Finally, the results of this project offer an opportunity to cooperate with enterprises to promote and replicate the production of newly developed rice varieties and produce rice seeds.

Keywords: *Rice, Vietnam, high-grade variety, National Rice Product, cultivation practices.*

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1. INTRODUCTION

The development of rice production is one of the most significant successes of Vietnamese agriculture after over 40 years of restoration. Rice cultivation contributes significantly to hunger eradication and poverty alleviation, providing food security and enhancing exports, foreign exchange earnings, and international relations [1].

Despite considerable gains, rice production still has several restrictions. Rice production has grown mostly in terms of breadth (acreage and productivity), whereas rice qualities and post-harvesting technologies are still low and limited, respectively. Furthermore, rice farming consumes significant resources, particularly water, along with fertilizers and pesticides frequently employed to boost yields, leading to resource inputs that can have environmental effects [2]. Despite ongoing advances in rice productivity, Vietnamese rice farmers have low income and considerable risks as compared to other value chain actors [3, 4].

To improve the efficiency of rice production, ensure a stable income for Vietnamese rice farmers, and develop sustainably, the Ministry of Agriculture and Rural Development issued Decision No. 3001/QD-BNN-KHCN dated July 28, 2015 [5] and Decision No. 1261/QD-BNN-KHCN dated April 12, 2016 [6] approving the Project: "Breeding and production of high-grade rice varieties, and development of advanced rice cultivation techniques for high productivity, high quality" under the National Product Development Programme to 2020 (called as National Product Program). Particularly, the Vietnam Academy of Agricultural Sciences is the principal agency for the implementation period 2016-2020, with a total budget of 269,030 billion VND (Vietnam's state budget: 104.25 billion VND and counterpart budget: 164.78 billion VND). The project encompasses eight tasks: one project for rice breeding, three projects for the development of rice production practices for three regions, including the Red River Delta, the Central Coast and the Mekong Delta, and four projects for experimental testing.

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2. OBJECTIVES OF THE NATIONAL RICE PRODUCT

2.1. Overall objectives

The overall objective of this project is to actively engage in the breeding of rice varieties with high quality, high yield, and high commercial value. Also, the development of sustainable rice cultivation practices to improve value-added, lower input costs, minimize greenhouse gas emissions, and boost rice growers' production and profit.

2.2. Detailed goals

- Construction of 4 - 6 new short-duration rice varieties with high yields and good quality.
- Construction of 3 - 5 aromatic rice varieties with short duration, high yield, and good quality.
- Cooperation with enterprises to produce and consume rice, construct typical fields and material-producing areas, ensuring the 2020 plan of achieving 2.0 million hectares per year by using certified seeds (reaching 12 million tons paddy per year) focused in the Red River Delta, the Central Coast, and the Mekong Delta.
- Production of pre-basis rice seeds (at least 35 tons per year), regietered rice seeds (3000 tons per year) and certified rice seeds (130,000 tons per year) for market demand.
- Development of a model for the production of high-grade rice grains and advanced cultivation practices for meeting domestic demand, as well as exporting high-quality rice.
- Construction of sustainable rice cultivation practices (seeds, fertilizers, pesticides and irrigation systems) to decrease prices, reduce greenhouse gas emissions, maintain food safety, and safeguard the environment in the major rice cultivation areas.
- Certification of new rice varieties and development of cultivation practices, and transmission of innovative and sustainable rice farming technologies to firms to increase rice production.

3. MAIN ACCOMPLISHMENTS

3.1. Reports of the project to select and develop rice cultivars with high economic value for the main growing regions

3.1.1. Reports of the rice breeding work

- A total of 11 new rice varieties have been developed from genetic materials. Particularly, six (OM20, OM429, OM375; OM402, OM10636, and BĐR27) and five (HD11, GL516, GL97, VN20, and DH12) rice varieties are generated in the Southern and Northern Vietnam, respectively, with the criteria of yield, quality, resistance to major pests and diseases, and adverse environmental conditions [7, 8, 9, 10, 11, 12]. Among them, five (GL516, ĐH12, OM429, OM402 and OM20) and three (HD11, OM375, and BĐR27) high-quality and aromatic rice varieties, respectively, have been legally approved for commercial release [7, 8, 9, 10, 11, 12].
- A total of five new promising rice lines, including OM08, OM469, OM449, OM476 and BĐR57 are generated to meet market demand. These promising rice lines have been evaluated for further approval.

3.1.2. Reports of the development and production of rice varieties

- ĐH12 rice variety: Since November 2018, Apollo Vietnam Investment and Development Joint Stock Company has had a license for the ĐH12 rice cultivar. The ĐH12 variety shared similar agronomical traits with the KD18 rice variety, such as wide adaptability, decreased susceptibility to pests and diseases, high and reliable yield, and delicious soft grain. The ĐH12 variety was then supplied as the major production variety structure in the Spring season of 2020. The ĐH12 rice variety has been distributed in the growing areas of 30 provinces in the Northern provinces, North Central Coast, and Mekong River Delta as of 2021, with an estimated area of over 80,000 hectares [7, 8].
- OM429 rice variety: By 2021, OM429 rice variety has been planted on approximately 6,000 - 8,000 hectares in the mekong river delta and southeast region of Vietnam. the OM429 variety is highly adaptable, capable of tolerating salinity of 4 - 6‰, and exhibits good rice quality with a high head rice percentage (58 - 64%). This variety exhibits potential expansion of cultivation in the Mekong Delta. the cultivar was transferred to Loc Troi Agricultural Research Institute on June 15, 2021 (Contract no. 08/HD/CGCN-VL).
- BĐR27 rice variety: BĐR27 variety was transferred the exclusive right to exploit and trade the original type and certified to Nong Viet Phat Company Limited on 10 January 2021 under Contract No. 01/ HDBQ. This rice variety has been planted on approximately 1,500 hectares in the South Central

Coast of Vietnam.

- GL516 rice variety: GL516 variety was transferred to Asian Queen Joint Stock Company on 25 December, 2020 under Contract No. 90/HD-CGBQ-VCLT). This variety has been planted on approximately 5,000 hectares in the Northern provinces of Vietnam.

- OM375 rice variety: OM375 variety was transferred to Nha Ho Seed Joint Stock Company on September 16, 2016 under Contract No. 39/2016/HD-CGCN/VL. As of 2021, the OM375 rice variety has been cultivated on around 700 hectares in the Mekong River Delta and Southeast region of Vietnam. This rice variety is highly adaptable and exhibits good resistance to blast disease (point 2), and hardiness (grade 1).

- OM20 rice variety: OM20 variety was transferred to the Nha Ho Research Institute for Cotton and Agricultural Development on May 18, 2018, under Contract No. 18/2018/HD-CGCN/VL. This variety has been cultivated on approximately 700 hectares in the Mekong River Delta and the Southeast region of Vietnam.

- OM08 rice variety: OM08 variety is a fragrant and high-quality cultivar. This rice variety is capable of salinity tolerance of 2 - 3‰. The OM08 rice variety canbe cultivated in three seasons in the Mekong Delta under saline-sodic conditions. The rice quality is comparable to that of the ST25 rice variety.

- HD11 rice variety: HD11 is a fragrant and high-quality rice variety. On July 19, 2020, the variety was certified by the Department of Crop Production under Protection Title No. 28.VN.2020. On October 14, 2019, theHD11 rice variety was transferred to Ha Noi One Member Agricultural Development and Investment Company Limited (Contract No. 65/HD-CGBQ-VCLT) [12]. This variety has been planted on approximately 7,000 hectares in the Northern provinces of Vietnam.

3.2. Reports of the project to establish advanced cultivation practices

3.2.1. Advanced cultivation practices for the Red River Delta

The project has absorbed and integrated concurrently advanced cultivation practices based on summarizing the recent rice farming approaches. These cultivation practices include (i) theapplication of *Trichoderma* spp. to suppress fungal infections and increase the decomposition of rice straw before plowing; (ii) the use of certified seed: pathogens are clearly treated before sowing, as well as the quality of substrates is improved by maintaining pH values and extending seedling shelf life without adding nutrients; (iii) sowing: Transplanting machines are highly recommended; (iv) pesticide application: Using a pressure sprayer with a 20-meter width; (v) fertilizer application: Slow-release fertilizers and fertilizer spraying machines are noticed tobe in use; (vi) irrigation: Water-saving irrigation techniques are applied; and (vii) harvesting: Combined harvesters are used to harvest in the fields.

The application of these advanced cultivation practices could reduce nitrogen by 25-30 kilos N per hectare, phosphorus by 35-40 kilos P_2O_5 per hectare, and potassium by 40-50 kilos K_2O per hectare. Using machinery to sow trays could reduce costs by 2.770 - 3.250 million VND per hectare, while spraying pesticides by machine could save between 2.438 and 2.865 million per hectare. In summary, using the whole package of advanced cultivation practices could significantly reduce production costs by an average of 4.818 - 7.966 million VND per hectare, raising economic efficiency from 17.5 to 36.9%.

According to a survey by the Department of Agriculture and Rural Development and the Center for Agricultural Extension, this advanced technological package has been comprehensively used in six provinces and cities, including Hai Duong, Ha Noi, Bac Ninh, Nam Dinh, Thai Binh, and Ninh Binh, with an entire cultivation area of 6,500 - 7,000 hectares per season. The model's productivity increased by 10-15% due to lower investment expenditures (reduced seed quantity from 10-12 kilos per hectare, fertilizer from 12-45%, pesticide use, and manpower for maintenance). Economic efficiency increased by 25-40% as compared with control.

Under proper conditions, the models are advised to use advanced rice cultivation practices, such as tray plating, transplanting machine, machine sowing, spraying, fertilizer application, and water saving. Fertilize and spray in accordance with the manufacturer's instructions, such as applying slow-release fertilizer, NPK Agrotain fertilizer, and spraying "4 Right".

To sum up, the technical package was acknowledged as a Technical Progress under

Decision No. 321/QD-TT-CLT dated 11/12/2020 [13].

3.2.2. Advanced cultivation practices for South Central regions

The proposed solution for rice cultivation practices in the South Central region of Vietnam has reduced the amount of rice seeds by 50%, from 120 to 60 kilos per hectare, nitrogen fertilizer from 28 - 37 kilos N per hectare (equivalent to 22.5 - 27.8%), spraying times and irrigation water in each season by about 30% and production costs by 2.851 - 3.882 million VND per hectare (equivalent to 10.13 - 12.00%). Yield increased from 479 - 546 kilos per hectare (equivalent to 6.9 - 8.0%) as compared to the control. The average expected profit is approximately 22.960 - 23.219 million VND per hectare, which is greater than the control of 6.482 to 6.495 million VND per hectare (equivalent to 38.8 - 39.3%). Furthermore, these rice cultivation practices are being used to lower CH_4 and N_2O emissions from 20.77 to 23.55 kilos per hectare per season and from 0.99 to 1.17 kilos per hectare per season, while total CO_2 emissions dropped from 814.3 to 937.4 kilos per hectare per season when compared to traditional farming.

Similarly, the proposed solution of rice cultivation practices for the North Central region in Vietnam has reduced the amount of rice seeds by 5 kilos per hectare, nitrogen fertilizer by 14 kilos N per hectare (equivalent to 12.7%), irrigation water in each season by over 30% and production costs by 1.8 - 2.6 million VND per hectare. Yield increased from 719 - 797 kilos per hectare (equivalent to 11.6 - 12.14%) as compared to the control. The average expected profit ranged between 29.635 and 30.225 million VND per hectare, which was greater than the control of 8.203 to 8.652 million VND per hectare (equivalent to 36.43 - 41.23%). The advanced technical package aids in lowering CH_4 and N_2O emissions from 98.22 to 117.09 kilos per hectare per season and from 1.08 to 3.04 kilos per hectare per season, while total CO_2 emissions are lowered from 2,776 - 3,834 kilos per hectare per season as compared to traditional farming.

The Department of Agriculture and Rural Development and the Center for Agricultural Extension did a survey that showed, that advanced technological package has been comprehensively used in four provinces, including Thanh Hoa, Nghe An, Quang Nam and Binh Dinh, with the entire cultivation area of approximately 2,200 hectares per season. Meanwhile, the proposed solution to rice cultivation practices for the South Central region of Vietnam has reduced the amount of rice seeds by 40 - 50%, irrigation water in each season by 30%, and pesticides by 1 - 2 times per season. Yield increased by 8%, while economic efficiency increased by 20% as compared with control.

To sum up, the technical package was acknowledged as a Technical Progressunder Decision No. 23/QD-TT-VPPN dated 25/01/2021 [14].

3.2.3. Advanced cultivation practices for the Mekong River Delta

Apackage of advanced rice farming practices has been performed in four sub-regions in the Mekong River Delta, including the three-season alluvial ecological sub-region (Cho Moi, An Giang), the two-season alluvial ecological sub-region (Thoi Lai, Can Tho), the alkaline soil ecological sub-region (Long My, Hau Giang), and the saline soil ecological sub-region (Tran De, Soc Trang). Economic efficiency of this package increased by 25-40% as compared with traditional farming. The technological packages aim to restrict greenhouse gas emissions (CH_4 and N_2O) and the influence on soil nutrients in each ecological region, in addition to lowering input prices. After one week of rice harvest, the rates of CH_4 and N_2O emissions in all of the advanced rice crops in the four eco-regions were lower than the average values in the old-fashioned fields for the period. The new model reduces CH_4 gas emissions by 66.6 - 93.9% and N_2O gas emissions by 15.9 - 80.6%; total greenhouse gas emissions converted to CO_2 are lowered by 8 - 9% compared to farmers' farming methods.

Particularly, according to a survey by the Department of Agriculture and Rural Development and the Center for Agricultural Extension, this advanced technological package has been comprehensively used in four provinces, including Can Tho, An Giang, Soc Trang, and Hau Giang, with an entire cultivation area of approximately 3000 hectares per season. Economic efficiency of this package increased by 25-41% as compared with traditional farming. The proposed solution of rice cultivation practices for these regions has reduced the amount of rice seeds by 80 - 120 kilos per hectare, nitrogen fertilizer by 20 - 40 kilos N per hectare (equivalent to 18 - 45%), phosphorus fertilizer by 20%, potassium fertilizer by 23%, irrigation water in each season by 3 - 4 times, and pesticide use by 2 - 4 times.

Overall, under proper conditions, the models are advised to use advanced rice cultivation practices. It could reduce the certified rice seeds by 80 - 120 kilos per hectare per season (equivalent to 30 - 50%), water use by 3 - 4 times per hectare per season (equivalent to 30%), fertilizer and pesticide use by 20 - 30%. Cutting between the rice stalks at harvest time reduces loss by 10-14% and boosts overall rice yield by 5-12% compared to conventional cultivation practices in all regions.

The technical package was acknowledged as a Technical Progress Under Decision No. 24/QĐ-TT-VPPN dated 25/01/2021 [15].

3.3. Reports of the project to produce high-grade rice varieties for production

3.3.1. Japonica rice production in Northern provinces in Vietnam

The initiative produced pre-basis *Japonica* rice varieties in Ha Noi, Ha Giang, and Phu Tho across an area of 8.5 hectares, yielding 26.8 tons of pre-basis rice seeds (reaching 89% in volume) that met the QCVN 01-54: 2011/BNNPTNT standard [16]. For the basis rice variety, production was arranged on a 60-hectare scale in the provinces and cities, including Ha Noi, Thanh Hoa, Yen Bai, Ha Giang, and Phu Tho, with a total production of 321.1 tons (reaching 53.5% in volume). The majority of the basis seed batches were used by businesses as a source of certified seed production to provide the locality. During the Spring season 2020, the project continued to deploy seed production models (joint enterprises) and commercial *Japonica* rice production models in Ha Giang, Vinh Phuc and Thanh Hoa provinces.

3.3.2. Rice production in the Northern provinces and South Central regions in Vietnam

The project has completed the industrial-scale validation breeding procedure for rice varieties BT09, LTH31, QP5, and Dong A1 (varieties that have been formally acknowledged by the task's contents). The project is expected to produce 57.35 tons of pre-basis rice seeds and more than 2,611 tons of basis rice seeds by the end of 2020, offering all cultivation areas of certified seeds on 20,000 hectares in the Northern provinces and South Central Coast in Vietnam.

3.3.3. Rice production of the North Central regions in Vietnam

This project has produced 47 tons of pre-basis rice seeds that were standardized by the Vietnamese quality standards (QCVN 01-54: 2011/BNNPTNT) [16], as well as 2,500 tons of basis rice seeds and 50,000 tons of certified seed for cultivation in the North Central region in Vietnam. By the end of 2020, the project will have completed 100% of the ordered seed production volume at all stages.

Furthermore, the project is implementing the supply and installation of machinery and equipment to meet the demands of seed processing, including 01 industrial-scale seed processing line with a capacity of 2.5 tons per hour, 01 seed testing and inspection laboratory to meet current regulations and 01 cooling system for preserving rice varieties. The contents of the construction and installation were finished and placed into operation in the fourth quarter of 2020, satisfying 100% of the anticipated content and volume.

3.3.4. Rice production in the Mekong Delta in Vietnam

The seed production and intensive farming process for rice types suitable for the region, such as OM18, OM9582, OM22, OM9921, and OM20 rice varieties, has been finished. Two fragrant rice cultivars, OM18 and OM9921, achieve the highest average yield in all three ecological sub-regions in the Mekong Delta. Furthermore, the project determined the best fertilizer formulary, harvesting times for each rice variety, as well as the processing quality of each rice type. The initiative organized the production of pre-basis rice seeds over a 20-hectare area, yielding more than 70 tons of seeds that met QCVN 01-54: 2011 criteria [16]. In terms of basis rice variety production, the project has collaborated with a number of units and businesses to organize 520 hectares of joint production models of basis rice varieties, with a total basis seed output of more than 2,800 tons.

3.4. Construction of anadvanced production model for high-grade rice varietiy cultivation

3.4.1. Construction of anadvanced production model for high-grade rice variety cultivation in the Red River Delta

The small-area testing showed that using slow-release fertilizer saves 22 - 50% of total fertilizer while still producing an 8 - 13% higher yield than the control in the Red River Delta. The use of mechanization can result in an average profit of 25 - 30 million VND per hectare, which is greater than the traditional conventional farming profit of 5 - 8 million VND per hectare (equivalent to 27 - 46%). Furthermore, when compared to traditional farming, the technology package reduces total emissions by 8.8 - 15.1%.

On that premise, it has been implemented on a broad scale with the collaboration of firms (Hung Cuc Limited Liability Company, Hong Quang Seed Joint Stock Company, Vietnam Seed Joint Stock Company, and Seed Joint Stock Company belonging to Field Crops Research Institute, with a total area of 160 ha in four ecological sub-regions in the Red River Delta. The economic efficiency of the models' rice production outputs exceeds 20.5 - 30.5% (Spring season) and 22 - 30% (Summer season) when compared to the control. In the near future, a technical package will be signed and transferred to these enterprises in order to implement commodity rice production organization on a wide scale, rapidly increasing the rice production area using innovative farming techniques. Farmers in the Red River Delta will benefit from this.

3.4.2. Construction of advanced production model for high-grade rice varieties cultivation in North Central regions

The concept was tested on a 40-hectare scale in Nghe An and Thanh Hoa provinces, and it lowered production expenses from 0.99 to 2.74 million VND per hectare (equivalent to 3.18 - 8.74%), raised output from 757-866 kilos per hectare (equivalent to 10.18-12.3%), and boosted profit from 6.78 - 9.25 million VND per hectare (equivalent to 28.4 - 30.2%). The amount of irrigation water has been decreased by 30% due to the use of the "alternating dry-wet" watering method; the model is used in conjunction with integrated pest control, thus the number of times pesticides have been sprayed has been reduced by 30%. In the case of pesticide use, the "4 Right" principle was applied to minimizie environmental contamination and toxicity to farmers. In addition, the entire production area has been used with combine harvesters in harvesting and drying, so the loss is reduced by less than 5%.

3.4.3. Construction of advanced production model for high-grade rice varieties cultivation in South Central regions

The concept was tested on a 40-hectare scale in Binh Dinh and Quang Nam provinces, and it lowered production expenses from 2.18 to 3.25 million VND per hectare (equivalent to 10.84 - 14.63%). The productivity was from 8.0 to 8.7 tons per hectare, slightly higher than the control. It has been boosted profit from 6.781 - 9.25 million VND per hectare (equivalent to 28.39 - 30.22%). The amount of irrigation water has been decreased by 30% due to the use of the "alternating dry-wet" watering method; the model is used in conjunction with integrated pest control, thus the number of times pesticides have been sprayed has been reduced by 30%. In the case of pesticide use, the "4 Right" principle was applied to minimizie environmental contamination and toxicity to farmers. Additionally, the entire production region has used combine harvesters to gather and dry seeds, reducing loss to less than 5%. The process's application area has been enlarged to over 1200 hectares in the South Central region (for the Northern and Southern sub-regions) by 2020.

3.4.4. Construction of advanced production model for high-grade rice varieties cultivation in Mekong Delta

The concept was tested in the Mekong Delta reveals that the amount of seeds has been lowered from 170-200 kg (control) to 80 - 120 kg of seeds (advanced model). It reduced the amount of fertilizer by 15 - 20%, the amount of pesticide uses by 900,000 to 1,850,000 VND per hectare, and water uses by 1-3 times per season as compared to the conventional methods. Furthermore, our results demonstrated that the amount of CH_4 and N_2O were greatly variable. The productivity ranged from 5.17 - 6.45 tons per hectare, whereas that in the control ranged from 5.06 - 5.97 tons per hectare, resulting in a better profit for the advanced model. In comparison to the conventional cultivation practices, the average profit increased by 1.0 - 1.6 million VND per hectare.

3.5. Cooperation with enterprises

3.5.1. Cooperation with enterprises to promote and replicate the manufacturing of newly developed rice varieties

The project's units have actively collaborated with firms operating in the field of rice seeds and agricultural materials in regions across the country since the project's inception. The strategy is that research units collaborate with enterprises beginning with the identification and selection of elite rice lines and continuing with the process of pure selection, testing, assessment, and the development of pilot production models.

Next, businesses can engage in developing promising rice varieties that meet their needs and business orientations in specific regions, so tying their interests to the development and expansion of varieties. When these businesses are permitted to begin production, they will be granted first priority in negotiating the transfer of seed use rights. In fact, up until the time of reporting, whole seven new rice varieties that had been approved for trial production/official release had worked closely with firms. Through this collaboration, new rice varieties are guaranteed to be promoted and introduced promptly and extensively through the seed distribution system of firms, making a vital contribution to achieving the criteria for area expansion.

3.5.2. Cooperation with enterprises to produce rice seeds

Production of Japonica rice varieties for the Northern provinces in Vietnam: Japonica rice varieties, such as J02, J01 and VAAS16 were cultivated by concentrated commodity chains in the Northern mountainous regions, Red River Delta, and North Central regions in Vietnam since the Spring 2020 season. The chairing unit has coordinated with enterprises such as Vietnam Seed Group Joint Stock Company and Vietnam Hi-Tech Company to develop large-scale models of japonica rice production on a size of 300-350 hectares for the Spring 2020 season.The models have been implemented from the stage of selecting a location, organizing the production material area, contracting with project participants, and training farmers on the technical process of intensive japonica rice cultivation with quality and efficiency, thereby ensuring japonica rice production and processing to meet export and domestic consumption needs.

Production of Indica rice varieties for the North and South Central provinces: A Indica rice varieties, including BT09, LTH31, QP5 and Dong A1 were cultivated at the industrial scale. The large sample field linkage model yields commercial rice at 66.7% area and 97.5% production. The model of linking large sample fields achieved 100% area and 111.9% yield for fragrant rice varieties.

Production of Indica rice varieties for the North Central region: Nghe An Agricultural Materials Corporation has finished the construction, installation, and testing of equipment as well as cold storage. In the Company's headquarters and the 6,000 m^2 rice milling and processing warehouse in Nghi Long Factory, Dong Nam Industrial Park, Nghi Loc district, Nghe An province, rice seeds are tested, processed, and preserved. Furthermore, the lead unit has implemented the development of model field linkage models based on the value chain at various sites in the North Central region, including the model of linking production of high-grade NA6 rice varieties with high quality standards. A 20-hectare scale in the spring crop of 2019 in Thach Dai commune, Thach Ha district, Ha Tinh province, a 450-hectare commercial rice production model in Thanh Hoa (200 hectares), Nghe An (200 hectares), and Ha Tinh provinces (50 hectares). In the models, the average yield was 6.5 - 7.0 tons per hectare in the Spring season and 5.3 - 5.6 tons per hectare in the Summer season. Spring season 2020 has deployed 5 models of large sample field linkages producing high-grade seeds (total area of 100 hectares) and 8 models of linking large sample fields producing commercial rice (total area of 400 hectares) in the provinces of the North Central region.

Production of *Indica* rice varieties in Mekong Delta: In the Mekong Delta provinces, the project is collaborating with the Seed Center, agricultural cooperatives, and the Department of Agriculture to deploy large sample fields to produce fragrant rice on a scale of 150 hectares and rice on a scale of 300 ha during the Spring season 2019-2020. The aromatic rice variety OM18 yielded 6.8 tons per hectare, whereas the high-quality rice variety OM9582 yielded 7.2 tons per hectare. In the 2019-2020 Spring season, the project was implemented on a scale of 150 hectares for the fragrant rice model and 300 hectares for the high-quality rice model in the next seasons in 2020. The models have been deployed from the stage of selecting a place, organizing the production material area, contracting with farmers participating in the project, and training those farmers on the technical process of commodity rice intensification with high efficiency and excellent quality rice varieties, assuring the production and processing of rice output to satisfy the requirements of export and home consumption.

4. SHORTCOMINGS, GAPS IN SCIENTIFIC AND TECHNOLOGICAL RESEARCH AND LESSONS LEARNED

Aside from the aforementioned accomplishments, Vietnam's rice business still faces numerous challenges. Export prices and competitiveness remain low, failing to fulfill the diversified needs of domestic and international markets; production and business efficiency remain poor, failing to match potential and advantages. Farmers' incomes are low and inconsistent, and there is no harmonization of interests across participants in the value chain. Rice production development is not sustainable; there are still many hazards in production and business, such as polluting the soil and water environment, damaging biodiversity, and being insensitive to the consequences of climate change.

4.1. Issues during the production stage

- Smallholder farmers' production scale is modest, and farmer association organizations (cooperatives) and farmer representation organizations have not been interested in development.

- Production relies on experience, a lack of scientific and technological information, and a lack of a market; young workers are leaving for other occupations; the level of intensive farming varies between areas, regions, and households within the same production area.

- Production is not in accordance with market demands; various types are grown in the same field, resulting in low and inconsistent quality of exported rice batches. The export rice variety set is still primarily aimed at markets that are simple to compute and sell at low prices. There are no fragrant, high-quality rice cultivars comparable to Pakistan's and India's Khao Dawk Mali 105 (Hom Mali) or Basmati. The majority of the known variants are resistant to brown planthoppers, blight, and rice blast.

- Input material quality (fertilizers, pesticides, seeds) has not been adequately managed; excessive use of fertilizers, pesticides, and seeds raises costs and pollutes the environment. The incidence of using certified seeds in the Mekong Delta is low, at roughly 15%, and the volume of seeds utilized is excessive (150-180 kilos per hectare).

- The use of good agricultural practices in production (IPM, 3 decrease -3 rise, 1 right - 5 decrease, VietGAP...) is still restricted, with inadequate attention paid to food safety and environmental protection.

- Rice monoculture, failure to pay attention to crop rotation, altering crop structure on rice land in order to improve efficiency, income, minimize pest and disease pressure, and lessen strain on rice consumption and export.

4.2. Issues during the post-harvesting process

- A lack of drying systems, particularly in the Mekong Delta's summer season, is generating losses and lowering the quality of exported rice.

- Buying rice is primarily dominated by private traders; thus, there is no guarantee of quality and no creation of a brand.

- Stored as brown rice before whitewashing and polishing for export; therefore, rice quality degrades quickly.

- In-depth processing, diversification of rice and rice-based products is restricted; no attention is paid to the utilization of by-products (rice husk, bran, rice straw, etc.) to boost added value and production efficiency.

4.3. Rice sector infrastructure and logistics are still restricted

The transportation and irrigation infrastructure have not been finished and do not meet production standards. The transportation system connecting manufacturing areas to the points of consumption and export is inadequate. Information systems and market ties are extremely restricted.

4.4. The shaky links between actors in production, purchasing, processing, and consuming

- Farmers and private traders (who collect over 90% of rice in the Mekong Delta), rice processing companies, and exporting enterprises are all part of the rice value chain. Unaffiliated rice, efficiently assisting one another for mutual advantage.

- Because most exporters acquire rice from private traders who are not affiliated with farmers to create vast fields and raw material areas, the quality of exported rice is low because firms collect from a variety of sources.

4.5. Policies have not met production and business requirements

- Small and vulnerable farmers; cooperatives play a limited role; enterprises mostly participate in the latter stage of the value chain, are unattached, and pay little regard to farmer issues.

- The Food Association does not yet represent all production and commercial actors along the rice value chain.

- Policy on land acquisition, credit, association support, and enterprise investment in agriculture is still limited and insufficient.

5. PRODUCT RESEARCH AND DEVELOPMENT ORIENTATION TO 2023

In order for the rice industry to continue to promote its existing advantages and maintain a strategic position in Vietnam's agricultural sector, it must continue to promote rice exports in the direction of raising prices, in addition to meeting the domestic market. Rice research, selection, and production not only assure food security in terms of quantity, but also of food safety and nutritional quality, contribute to community health improvement, and fits demands and tastes. Increasing consumer demand, environmental preservation, and climate change adaptation. To achieve the aforementioned objectives, the following specific solutions are available:

5.1. Rice breeding work

- Continuing to research and select high-quality, fragrant, high-priced rice varieties that are competitive in both export and domestic markets, are resistant to major pests and diseases, and prefer short-growth or high-quality aromatic mid-day lines.

- The Mekong Delta region is primarily focused on the export market, while also paying close attention to the domestic market; high-quality rice varieties (white rice, long grain, lightly fragrant or not fragrant) account for approximately 50% of the cultivated area; fragrant rice varieties account for approximately 25%; glutinous varieties, local specialties account for approximately 15%; and average-quality varieties account for approximately 10%.

- The Red River Delta and the remaining cultivation regions are mostly aimed at the domestic market; rice varieties with high selling prices and good quality rice are primarily used. They mostly focused on the high-quality rice varieties, such as glutinous rice varieties and japonica rice varieties, account for approximately 60% of the cultivation area, while high-yielding types account for approximately 40%.

5.2. Application of sustainable cultivation practices in rice production regions

- Establishment of policies of area planning to construct a large rice production area with synchronous infrastructure for commercial rice production.

- Upgrading, improving, and creating irrigation and electricity systems in the field to replace oil-based pumps.

- Using certified seeds (level 1 or level 2), the rate of use of certified seeds in Vietnam will exceed 95% by 2030.

- Prioritizing the use of organic fertilizers, rice-tailored synthetic NPK fertilizers; fertilize based on soil nutrition and rice demands; minimize fertilizer consumption by at least 30% over current levels.

- Implementing integrated pest management on the entire rice cultivation area; organize full-time plant protection services at the facility; rationally use herbicides, only use pesticides when necessary, and must adhere to the "4 rights" principles; reduce pesticide use by at least 30% compared to current levels.

5.3. Post-harvesting management

- Using high-tech combined harvesters to harvest rice in major cultivation areas, with a residual harvest of less than 1.5%.

- Converting the rice drying system from 2-step drying to 1-step drying using fluidized bed drying and tower drying. Drying fresh rice to the standard moisture content of 14.0 -14.5% in order to transition from preserving dry rice grains to dry rice seeds.

- Screening of the warehouse system (4 million tons capacity), balancing the demand for storing rice in specialized cultivation regions in order to construct and upgrade the rice storage system with drying, cleaning, and automation systems.

- Assistance with land, funding, and market access for cooperatives and allied businesses in important specialized farming areas to construct dry rice storage facilities.

- Assisting businesses in purchasing advanced processing technology, such as deep processing. Creating a direct trading platform for business-to-business transactions to exchange processing technology.

5.4. Prevent the effects of climate changes

Report and constantly update climate change scenarios for rice production in Vietnam; design synchronous response solutions for each scenario, focusing on the following solution groups:

- Develop and implement technical packages to adapt to climate change: alter the season, adjust the cultivation regions, develop and implement resistant rice varieties, and implement appropriate cultivation practices, like rotation, multi-cropping and intercropping to reduce risks and capitalize on natural advantages.

- Research and implement strategies to reduce greenhouse emissions and mitigate the effects of climate change: rationally manage water use to save water for rice production, reduce agricultural material consumption.

- Implement production planning and shift population clusters in response to climate change scenarios.

- Invest in constructing a protection forest system and building infrastructure, including irrigation systems and roads, to adjust to climate change.

5.5. Risk management

- Creating an information system for proactive warning, weather forecasting, natural disaster prevention and control, and epidemic prevention and control.

- Implement a rice agricultural production insurance program (support and encourage farmers to purchase insurance, and encourage insurance companies to engage in the market).

- Financial, technical, and in-kind assistance to rice farmers in the event of catastrophic natural catastrophes to restore production and stabilize livelihoods.

- Investigation of rice varieties that are resistant to major pests and diseases, and adverse environmental conditions. Application of eco-friendly rice farming to minimize the impacts of pests, diseases and climate changes. Also, to establish a dependable, pest-resistant, disease-resistant, and disaster-resistant seed supply system in each production region.

- Implement policies to encourage the use of integrated pest management, sustainable farming to provide a stable ecological environment, and the prevention of pesticide resistance.

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